COMPARATIVE EVALUATION OF DEPTH OF PENETRATION OF NANOSILVER AND CHLORHEXIDINE INTO THE DENTINAL TUBULES IN ROOT CANAL - AN INVITRO STUDY

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Towards the partial fulfillment for the degree of

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BRANCH – IV

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Certificate

This is to certify that **Dr. SAUJANYA KUMARI .K.P,** post graduate student (2007-2010) in the Department of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital,Chennai-3, has done this dissertation titled **"COMPARITIVE EVALUATION OF DEPTH OF PENETRATION OF NANOSILVER AND CHLORHEXIDINE INTO THE DENTINAL TUBULES IN ROOT CANAL – AN INVITRO STUDY"** under our direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai-32** for **M.D.S.** Conservative Dentistry and Endodontics (Branch IV) Degree Examination.

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TITLE OF DISSERTATION	**COMPARATIVE EVALUATION OF DEPTH OF PENETRATION OF NANOSILVER AND CHLORHEXIDINE INTO THE DENTINAL TUBULES IN ROOT CANAL – *An Invitro Study***
PLACE OF THE STUDY	**TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL, CHENNAI-3.**
DURATION OF THE COURSE	**THREE YEARS**
NAME OF THE GUIDE	**DR. S.C. LOGANATHAN**
HEAD OF THE DEPARTMENT	**DR. S.C. LOGANATHAN**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance / any promotion without obtaining prior permission of the Principal, Tamilnadu Government Dental College and Hospital, Chennai-3. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to preserve for publish of the work solely with the prior permission of the Principal, Tamilnadu Government Dental College and Hospital, Chennai-3.

Head of the Department

Signature of the Candidate

Contents

Introduction

INTRODUCTION

Bacteria play an essential role in the initiation, progression and persistence of apical periodontitis and pathology[28,37]. Therefore, endodontic therapy aims to eliminate bacteria from the infected root canal and prevent infection.

Cleaning through irrigating & shaping of the root canal greatly reduces the number of bacteria but doesn't completely eliminate them[7]. Concern exists not only to the consequence of bacteria left in the root canal but also to those micro organisms that remain in the dentinal tubules[42,46].

Invitro studies have shown that bacteria are able to penetrate dentinal tubules of the root upto 800μm, when the cementum is removed from the root surface and the smear layer from the root canal wall[42,23,53,45].

Ando and Hoshino[2] reported the presence of viable bacteria in dentin samples collected from areas at a distance of 0.5 to 2mm from the canal-dentin boundary of infected teeth with heavily decayed clinical crowns.

In hiotological sections of infected roots, **Armitage at al**[3]. found bacteria in the dentinal tubules half-way up to the cementodentinal junction.

Sen et al[59,47] found bacteria and yeasts in the dentinal tubules of extracted teeth in a range from 10 to 150μm using scanning electron microscopy.

Persistent infection within the root canal and periapical area is a source of concern in endodontics. Inadequate disinfection of the infected root canal and associated periapical lesion can lead to persistance of the infection. Failed root canal treatments have been attributed to viable bacteria that exists within the root canal & periapical system[51,70].

It has been hypothesized that these viable bacteria within the root canal system and dentin tubules can be a source of reinfection or continued periapical inflammation[54]. To prevent reinfection of a treated root canal, it is important to disinfect the pulp space & dentinal tubules thoroughly with an endodontic irrigant or intra canal medicaments.

Important requirements of an endodontic irrigant include properties such as antimicrobial activity, tissue dissolving property and nontoxicity to the periapical tissues[10,19]. There have been many studies performed to determine which root canal medicines and irrigants are most effective. Most commonly, the irrigants studied were sodium hypochlorite and Chlorhexidine(CHx)

Despite the use of many irrigants and intercanal medicaments like Calcium hydroxide[$Ca(OH)_2$], certain microbial species do survive and cause persistent infections. Enterococcus faecalis has long been implicated in

persistent root canal infections. It has been recently identified as the species which is most commonly recovered from root canal with post treatment periapical infections[56].

Even under starved condition E.faecalis shows resistance to sodium hypochlorite. E.faecalis can proficiently invade dentinal tubules surviving chemomechanical instrumentation & intracanal medication colonizing the tubules and reinfecting the obturated root canal[36].

E.faecalis plays a major role in root canal failure cases as it can invade into the dentinal tubules which is inaccessible for any chemomechanical disinfection or host defence mechanism. Several studies have reported that sodium hypochlorite, Ethylene diamine tetra acetic acid (EDTA), Calcium hydroxide are ineffective on E.faecalis. It has been shown that, at present 2% Chlorhexidine is effective in the elimination of E.faecalis from dentinal tubules.

Silver is a non-toxic, safe inorganic antibacterial agent used for centuries & is capable of killing about 650 types of organisms[27]. Silver ions and silver based compounds including silver Nanoparticles are highly toxic to microorganism but have low toxicity towards host cells[50]. Recently efficacy of silver Nanoparticles have been studied on E.faecalis and found that Nanosilver is effective on E.faecalis.

Depth of penetration of sealers & evaluation of irrigants have been studied indirectly in many previous studies.

In this study an attempt have been made to evaluate the penetrating ability of the two irrigants. i.e., 2% Chlorhexidine and Nanosilver solution into the dentinal tubules in root canal.

Aims and Objectives

AIMS & OBJECTIVES

The aims and objectives of the present study are :

1) To synthesize Silver Nanoparticles

2) Characterization of silver Nanoparticles by TEM analysis

3) To evaluate depth of penetration of Silver Nanoparticles solution and Chlorhexidine into the dentinal tubules using Confocal Laser Scanning Microscope.

Review of Literature

REVIEW OF LITERATURE

Enterococcus Faecalis

Bacteria in root canals may contribute an important reservoir from which root canal infection occur following pulp necrosis during & after endodontic treatment (**Oguntebi 1994**)[40]. He concluded that ***newer treatment strategies that are designed to eliminate this microflora must include agents that can penetrate dentinal tubules & destroy them,*** since they are located in area beyond host defense mechanism or the action of antimicrobial agents.

Variation in the susceptibilities of endodontic microflora to chemical procedures was studied by **Gomes et.al, 1996** [(20)] & confirmed that organisms like E.faecalis were recovered from canal after thorough biomechanical preparation. He concluded that certain organism like E. faecalis are difficult to eliminate from infected root canals.

Love et at in 2001[(34)] postulated that virulence factor of E.faecalis in failed endodontic treated teeth may be related to the ability of E.faecalis to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum. E.faecalis virulence may also be related to its resistance to intracanal medicaments, adherence to host cells, expression of protein to ensure cell survival as a result of altered nutrient supply, ability to compete with other bacterial cells and alter host response & environment.

Vivaoqua et al in 2005[77] studied recovery of E.faecalis after single or multiple visit root canal treatments carried out in infected teeth Ex-vivo. He concluded that neither single nor multiple visit root canal treatment eliminated E. faecalis that is remaining viable inside the dentinal tubules.

E. faecalis associated with asymptomatic chronic periradicular lesions is significantly more than acute periradicular abscesses (**Charles H.Stuart et al.2006**[9]).

E. faecalis was found in 4 to 40% of primary endodontic infection (**Rocas et.al**). E.faecalis was also commonly associated with asymptomatic cases than with symptomatic lesions. Its ability to cause periradicular infections stems from its ability to survive the effect of root canal treatment and persist as a pathogen in root canal & dentinal tubules.

Irrigants:

It has been demonstrated that bacteria are the etiologic agents of pulpal necrosis and apical periodontitis. Because of the complexity of the root canal system, mechanical instrumentation cannot adequately remove bacteria and tissue from all root canal surfaces. In addition, mechanical instrumentation forms a smear layer on the canal surface. Thus, irrigation is required to remove debris, tissue remnants, microbes and the smear layer.

A combination of Sodium hypochlorite (NaOCl) and EDTA is an effective combination for removing both pulpal tissue and smear layer

(**Baumgartner J.C 1987**, **Yamada RS 1983**)[6,78]. NaOCl is effective at dissolving organic tissue and has antimicrobial property **(Senia E.S et. al**. **1971**)[62].

In order to obtain the maximum effect during and after instrumentation, it is necessary to use chelating agents in conjuction with a tissue solvent (**Yamada et al 1983, Baumgartner 1987**)[78,6].

However, there is a safety concern if NaOCl is extended out of the root canal apex and into the periapical tissues, resulting in destructive tissue damage (**Ehrich, 1993**).[18.] NaOCl also corrodes and weakens endodontic instruments and has a disagreeable odor (**Bauman MA 2007**). Thus, alternative irrigant such as 2% CHx gluconate have been considered.

An effective method to remove the organic and inorganic remnants is to irrigate the root canal with EDTA followed by NaOCl[(22)].

Chlorhexidine(CHx) has comparable antibacterial efficacy to NaOCl (**Jeans MJ et al 1994**)[26] and has the advantage of having substantivity (**Dametto et al 2005)**[12]**.**

Edgar.Schafer et al 2005[(16)] studied the antimicrobial efficacy of CHx and two calcium hydroxide formulations against E.faecalis. It was found that CHx was significantly more effective against E.faecalis than $Ca(OH)_2$ paste or a mixture of CHx with $Ca(OH)_2$ paste. There was no increase in the efficiency of $Ca(OH)_2$ paste when CHx was added. The results suggest that CHx is

effective in the elimination of E.faecalis from dentinal tubules (**Tung B. Bui 2008,Bettina Boorani et al 2003**).

Chlorhexidine has been suggested for use as an irrigant and intracanal medicament because of its lower toxicity when compared with NaOCl (**Yesilsoy 1995**)[79].

Chelating agents remove the smear layer from the root canal and potentially allow better dentinal tubule penetration of root canal irrigants and sealers as well as in demineralizing and softening dentine (**Sen et al 1995; Hulsmann et al 2003**)[61].

Siqueira and Milton de uyede in 1997[(65)] studied the disinfection of dentinal tubules with two obligate and one facultative anaerobic bacteria by $Ca(OH)_2$ pastes. Their study concluded that pH values reached by $Ca(OH)_2$ were insufficient to kill some bacterial strains like E.faecalis. E.faecalis can survive at a pH of 11.5 and can penetrate dentinal tubules.

However, CHx lacks the tissue dissolution capabilities of NaOCl. Hence, it has been suggested that CHx not to be a replacement irrigant to NaOCl but a supplemental final irrigation step after irrigation with NaOCl and EDTA (**Kuruvilla JR, Kamath MP et al 1998**)[31].

Chlorhexidine is a broad spectrum antimicrobial agent that disrupts the membranes of microbes (**Leonardo MR 1999**)[32].

Teixeira et al in 2005[71] studied the effect of application time of EDTA and NaOCl on intracanal smear layer removal. He concluded that canal irrigation with EDTA and NaOCl for 1, 3 & 5 min were equally effective in removing the smear layer from the canal walls.

Trisha et al 2007[74] compared the antimicrobial effect of MTAD, NaOCl, Doxycycline and Citric acid on E.faecalis. They concluded that NaOCl and Doxycycline were more effective in killing E.faecalis than MTAD. Citric acid penetrated to a depth of 100μm into the dentinal tubules. None of them were able to render the canal sterile and they suggested finding of an intracanal irrigant that can completely eradicate E.faecalis from root canal dentin.

Sedgley in 2007[57] evaluated that Biopure MTAD is a viable medicament against E.faecalis.

Daniel P. Oliveira et al in 2007[13] compared the invitro antimicrobial activity of 2% CHx against E.faecalis with NaOCl in 2 different concentrations. They concluded that 2% CHx and 5.25% NaOCl were effective in eliminating E.faecalis.

Silver Nanoparticles :

Nanoparticles displayed unique, superior and indispensable properties and represent an important class of material in the development of novel devices that can be used in various physical, biological, biomedical and

pharmaceutical applications. Their uniqueness arises specifically from higher surface to volume ratio.

Silver and gold Nanoparticles in combination with laser have been successfully used in photoablation of tumors.

Sondi and Salopek - sondi in 2004[67] investigated the antimicrobial activity of silver nanoparticles against E.Coli representing gram (–ve) bacteria. SEM and TEM analysis was done. The results confirmed that the treated E.coli cells were damaged showing formation of pits in the cell wall of the bacteria, while the Nanosilver were found to accumulate in the bacterial membrane and causing increase in permeability & resulting in death of the cell. They concluded that ***nontoxic Nanomaterials, which can be prepared in a simple and cost effective manner may be suitable for the formulation of new types of bactericidal materials.***

A review article on **Nanoscale materials (2006)**[39] states that silver is a ***broad spectrum antimicrobial agent***. ***Nanoscale silver*** has been recognized as a ***more potent antimicrobial form of silver.***

It was demonstrated that wound dressing coated with sputtered Nano scale silver reduced infection in burns. The antibacterial action of silver Nanoparticles is cell death due to uncoupling of oxidative phosphorylation **(Holt and Bard, 2005**). others report the interaction with membrane bound enzymes and protein thiol groups result in compromised cell wall integrity that

would have lead to deterioration of proton gradient driven oxidative phosphorylation.

Ales panacek et al 2006[1] successfully demonstrated the bactericidal effects of size controlled silver nanoparticles using one step modified Tollens process. Silver nanoparticles with an average size of 25nm showed high antimicrobial & bactericidal activity against gram +ve and gram –ve bacteria, including highly multiresistant strains such as methicillin resitant staphylococcus aureus.

A very low concentration of sliver as low as 1.69 μg/ml Ag gave antibacterial performance by an irreversible inhibition of bacterial growth.

Sukdeb et al in 2007[69] investigated the antibacterial properties of differently shaped silver nanoparticles against E.coli. They concluded that silver nanoparticles undergo a shape dependent interaction with the gram(–ve) bacterium E.coli. They speculated that silver nanoparticles with same surface areas but with different shapes may also have different effective surface areas in terms of active facets.

Siddhartha Shrivastava et al in 2007[64] characterized the antibacterial effect of silver nanoparticles. The antibacterial effect of silver nanoparticles was found to be more potent against gram(–ve) bacteria than gram(+ve) organisms and was dose dependent and acquisition of resistance by bacteria against antibiotics. The major mechanism through which silver nanoparticles

manifested antibacterial properties was by modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues.

Kim et al in 2007[29] prepared stable silver nanoparticles and their shape and size distribution were studied by particle characterization under TEM. The antimicrobial activity of silver nanoparticles was investigated against yeast, E.Coli and S.aureus. The result showed that yeasts and E.coli were inhibited at the low concentration of silver nanoparticles, whereas the growth inhibitory effects on S.aureus were mild. The free radical generation effect of silver nanoparticles on microbial growth inhibition was investigated by Electron spin resonance spectroscopy. Their results suggested that silver nanoparticles can be used as effective growth inhibitor against carious microorganisms making them applicable to diverse medical devices and antimicrobial control systems.

Raffi et al in 2008[50] characterised the antimicrobial activity of silver nanoparticles as a function of particle concentration against gram (–ve) bacterium E.Coli. SEM and TEM showed that silver nanoparticles after interaction with E.coli, it adhered and penetrated into the bacterial cells.

Antibacterial properties of sliver nanoparticles were attributed to their total surface area where increased surface to volume ratio of nanoparticles provided more efficient means for enhanced antibacterial property.

Eduardo et al in 2008[17] studied the antibacterial effect of silver nanoparticles against E.Coli (g-ve) and staphylococcus aureus (g+ve) organisms. The silver nanoparticles were found to exhibit a good bactericidal

activity against both gram (-ve) and gram (+ve) organisms but gram(+ve) organisms required a higher concentration to achieve bactericidal effect and also required a longer exposure time for antibacterial effect.

Materials and Methods

MATERIALS AND METHODS.

The following materials and Armamentarium are used in this study (Fig.1 to Fig.12)

MATERIALS

1. Freshly extracted teeth stored in 0.9% saline
2. 0.9% (V/w) Saline
3. RC prep-canal lubricant
4. 2.5% Sodium Hypochlorite
5. 17% EDTA solution
6. Distilled Water
7. 2% Chlorhexidine (CHx) (Dentochlor, Ammdent)
8. Silver nitrate (AgNO3) (Ranbaxy Las. Ltd. Sas Nagar, Punjab)
9. Sodium tricitrate and sodium borohydride (99% purity – Aldrich chemicals)
10. Propylene glycol (Fisher)
11. Rhodamine-B Fluorescent Dye
12. Self cure Acrylic, Modeling wax

ARMAMENTARIUM

1. Gloves & mouth mask
2. Gauze
3. Tweezer
4. Straight Hand piece (*NSK*)
5. Diamond disk
6. Glass Petri Dish
7. Endo Block
8. Hand K-Files (*Mani)*
9. Anthogyr gear reduction hand piece
10. Rotary protaper files *(Dentsply*)
11. TEM (*Hitachi HF 2000*)
12. Electronic Balance
13. Glass Beakers and stirrer
14. Micropipette
15. 27 Gauge irrigating needles (*Nipro*)
16. Hard tissue microtome *(Leica SP 1600*)
17. Glass slides and micro cover slips (*Blue Star*)
18. Confocal laser scanning microscope (*Leica TCS SP 2, Germany*)

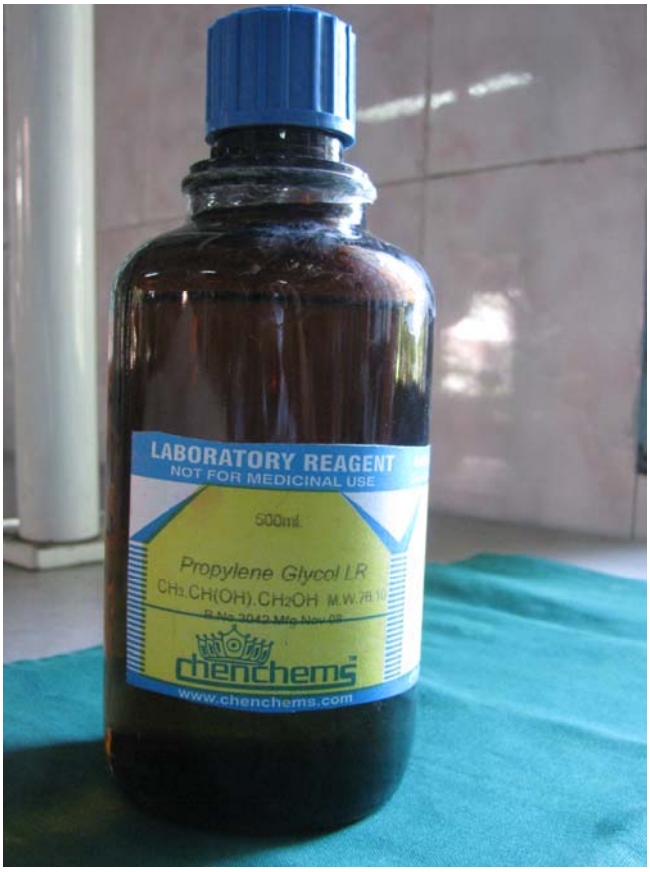
(Fig.1) Extracted Teeth Samples

(Fig.2) Armamentarium for Bio-mechanical preparation

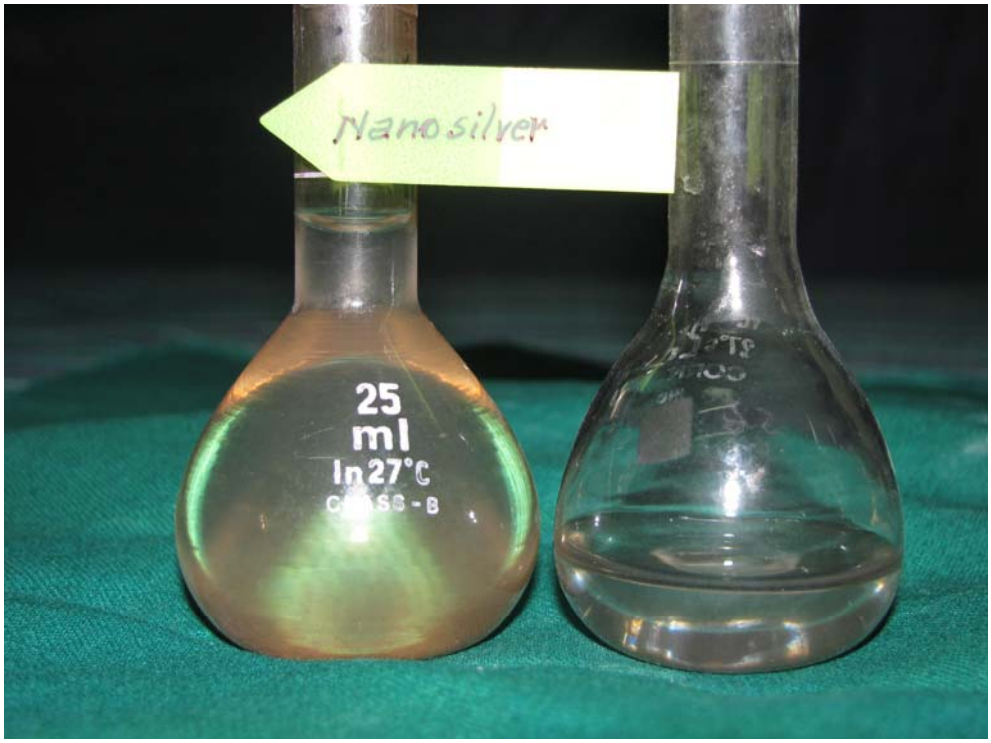
(Fig.3) 2.5% Sodium Hypochlorite

(Fig.4) 17% EDTA Solution

(Fig.5) 2% Chlorhexidine Solution



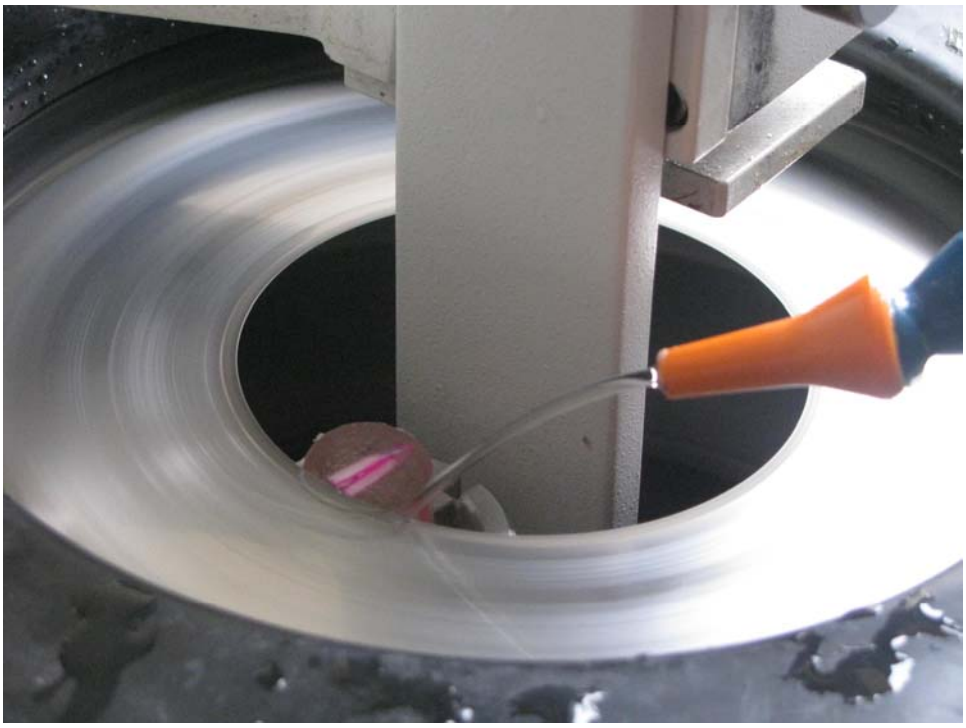
(Fig.6) Propylene Glycol



(Fig.7) Nanosilver Solution

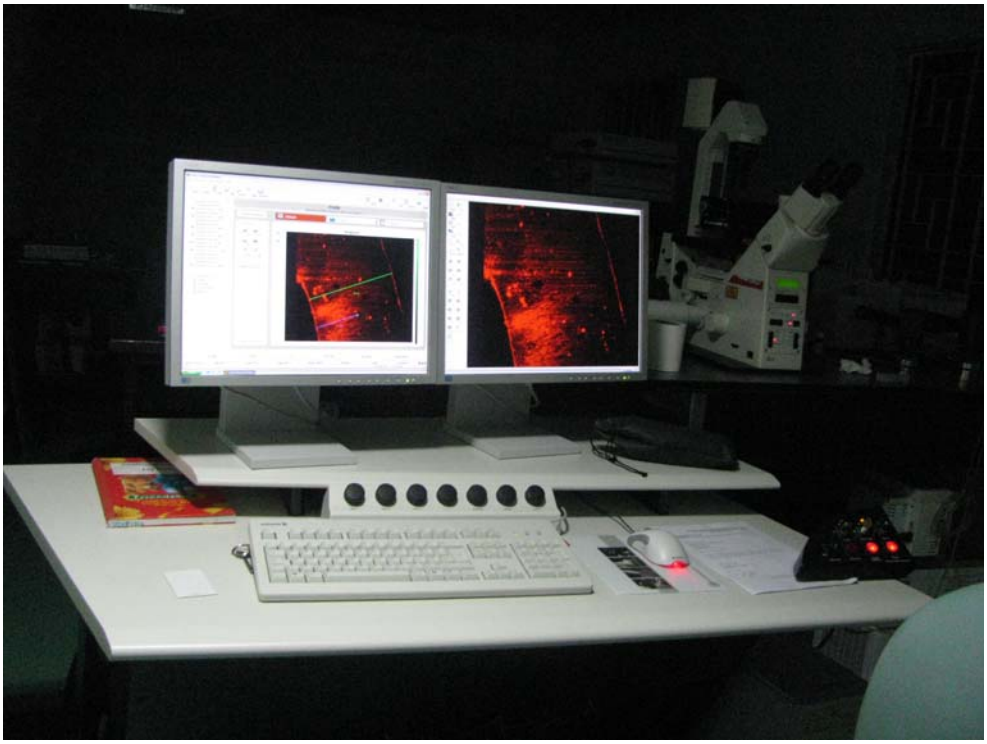
(Fig.8) Rhodamine B Powder

(Fig.9) Hard Tissue Microtome



(Fig.10) Close-up View of Hard Tissue Microtome

(Fig.11) Glass Slides & Micro-Glass Cover Slip



(Fig.12) Confocal Laser Scanning Microscope

METHODOLOGY

Step 1 : Synthesis of Silver Nanoparticle (SNP) solution

A solution containing 100 microgram of silver nanoparticles (Fig.13) in 1ml was prepared by adding 5ml of 10^{-4}M solution of $AgNO_3$ with 5ml of 0.1M sodium tri-citrate. The solution was brought to higher temperature of about 45^{O}C and stirred well. Then, a few drops of 0.1M sodium borohydride ($NaBH_4$) was added to it . The colour of the solution changed from Colorless to Yellow. While adding Sodium borohydride the solution was stirred very fast. After 30 minutes, the temperature was lowered to room temperature and then the solution was left with stirrer for growth process of homogenized sized silver nanoparticles.

(Fig.13) Silver Nanoparticle Solution

Step 2 : Characterization of Silver Nanoparticles

Characterization techniques play a vital role to study the materials inorder to understand the changes in their properties with respect to particle size. Various characterization techniques are employed to study the properties of nanoparticles. A brief description of the experimental technique employed in the present investigation on Transmission Electron Microscopy (TEM) is presented in this section. (Fig.14(a) & 14(b))

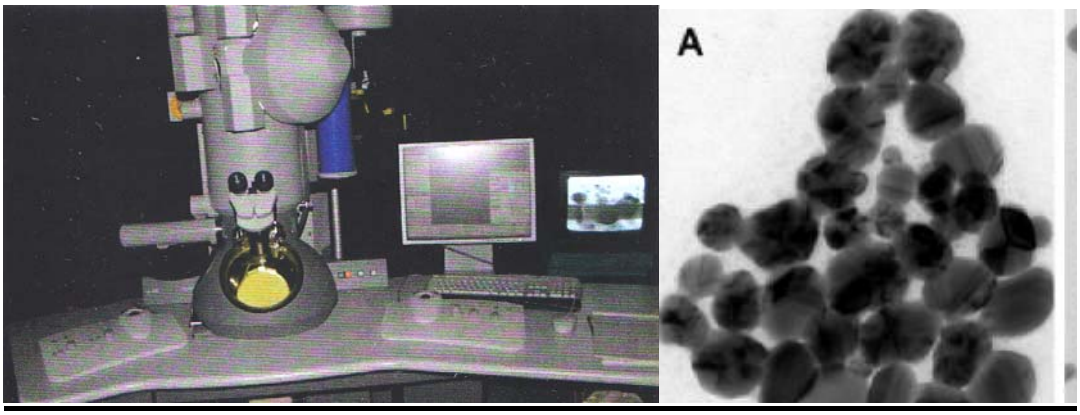


Fig.14 (a) TEM & Fig.14 (b) TEM image of Silver Nanoparticles at 30000x magnification

Transmission Electron Microscopy (TEM) :

The collected silver Nanoparticles were characterized by using Transmission Electron Microscopy (TEM) for estimation of crystalline structure, mean size and morphology. For TEM analysis, a drop of dilute solution of silver Nanoparticles was kept on the carbon coated copper grids and dried. The morphology and size of the Nanoparticle sample were analyzed

using Hitachi (HF-2000) Transmission Electron Microscope (TEM) with a resolution of 2-3Å

Transmission Electron Microgram (TEM) of Silver Nanoparticles

The diameter of more than 100 particles were measured. TEM micrograph of silver Nanoparticles suspension (100μg/ml) at 30000x magnification demonstrated agglomerated clusters and individually dispersed particles of spherical shape and narrow particle size distribution. The average diameter of Silver: citrate was determined to be 15 $\pm$ 0.3nm (Fig.14(b)). This silver : citrate nanoparticles were significantly smaller and better dispersed as compared with those in the literature. The size of the nanoparticles in general is determined by relative rates between nucleation and particle growth. The formation of the small silver nanoparticles can be ascribed to suppression of the growth of the silver nuclei under the present preparative conditions by the strong reducing agents sodium borohydride ($NaBH_4$) instantaneously. Production of large number of silver nuclei which grow into larger particles is retarded by the lower temperature of the medium.

Step 3 : Preparation of 0.1% Rhodamine B Solution and Conjugation with Irrigants

Rhodamine B fluorescent dye powder is quantitated to make 0.1m mol/0.1% Rhodamine B solution.

The formula used

$$1 \text{ m.mol} = \frac{10 \times \text{mg / dl}}{\text{Mol. Wt}}$$

$$\text{Mg/dl} = \frac{1 \text{ m.mol} \times \text{mol.wt}}{10} \qquad \text{(mol. Wt of Rhodamine = 479)}$$

$$= \frac{1 \times 479}{10}$$

$$\text{Mg/dl} = 47.9$$

Therefore 1m.mol = 47.9 mg/100ml

0.1m.mol = 4.79mg/100ml

To make 0.1m.mol concentration of Rhodamine solution 4.79mg of Rhodamine B is weighed using Electronic balance and dissolved in 100ml of experimental solutions (Fig.20, 21 & 22) like mentioned below-

- 4.79mg of Rhodamine B powder dissolved in 100ml of distilled water.
- 4.79mg of Dye in 100ml of 2% CHx

- 4.79mg of Dye in 100ml of Nanosilver solution
- 4.79mg of Dye in 90ml of Nanosilver solution and 10ml of
- Propylene glycol i.e. Nanosilver in Prophylene glycol is prepared in the ratio 9:1 by volume

Step 4:

Sixty straight single rooted, single canal, maxillary and mandibular teeth of young patients (age group of 15-40) extracted for the purpose of orthodontic and periodontal reason were selected for this study (Fig.15).

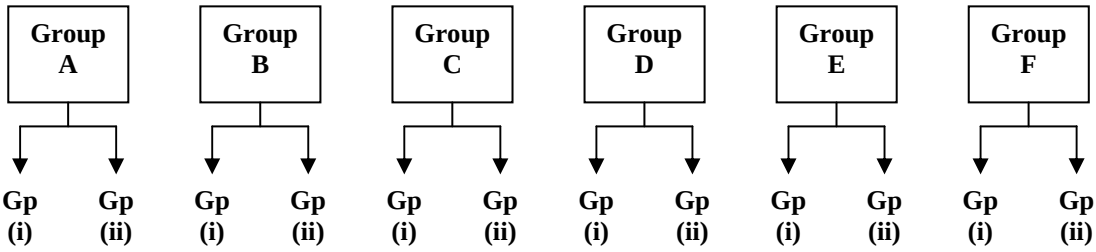
Exclusion Criteria for teeth selection includes :

- Carious Teeth
- Calcified Canals
- Sclerosed Teeth
- Bifurcated Canals
- Fractured Teeth
- Highly restored teeth

All teeth were stored in 0.9% saline. Teeth were decoronated at the cemento-enamel junction using diamond disk(Fig.16). Pulp was extirpated with barbed broach. patency of the canal checked using no.10 K-file(Mani). Working length was determined by using 10 size k files. Biomechanical preparation (Fig.17) of root canal was done using rotary protaper files(Dentsply Mallefer, Switzerland) according to manufacturer's instruction to follow the sequence

of file and at the recommended torque and speed in a crown down technique (Fig.18). Root canal preparation have been done upto protaper finishing file No.30 (F_3) at the apical 3rd. During each instrumentation RC Prep(Prime Dental) was used as lubricant. During change of each instrument recapitulation has been done with no. 15 k.file.

Before final irrigation all the root samples were embedded in wax blocks(Fig.19) and the samples were divided randomly into 6 main groups namely A, B, C, D, E & F (n=10 in each group) and each main group was further divided into two sub groups i.e. Gp(i) & Gp (ii).



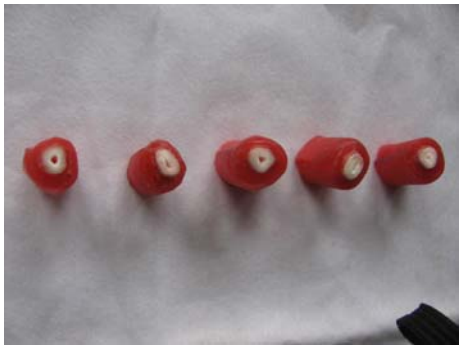
After grouping the samples, smear layer was removed. Smear layer was removed by using 2ml of 17% EDTA(Pulpdent, watertown, USA) for 1 min followed by 2ml of 2.5% NaOCl(Nice) irrigation using 27 gauge needle(Nipro) **(Teixeira cs et at 2005)**[71] in all the groups except group B.

(Fig.15) Extracted Teeth samples

(Fig.16) Decoronation of samples

(Fig.17) Bio Mechanical preparation with K file

(Fig.18) Bio Mechanical preparation



(Fig.19) Embedding samples in Wax blocks

(Fig.20) Preparation of 0.1 m mole Rhodamine B solution

FINAL IRRIGATION :

Final irrigation was done passively with 0.1% Rhodamine B dye(Sigma) dissolved experimental irrigants in each groups as shown below, using syringe and 27 gauge needle (Fig.23).

Group A (n=10) Positive control	Group (i) : (n=5)	10ml of distilled H_2O using 27 gauge needle for 3 min
	Group (ii) : (n=5)	10ml of distilled H_20 for 5 min
Group B (n=10) negative control	Group (i) : (n=5)	Smear layer retained + 10ml of distilled H_2O for 3 min
	Group (ii) : (n=5)	Smear layer retained + 10ml of distilled H_20 for 5 min
Group C (n=10) Positive control	Group (i) : (n=5)	10ml of aqueous solution of prophylene glycol for 3 min
	Group (ii) : (n=5)	10ml of aqueous solution of prophylene glycol for 5 min
Group D (n=10)	Group (i) : (n=5)	10ml of 2% CHx solution for 3 min
	Group (ii) : (n=5)	10ml of 2% CHx solution for 5 min

Group E (n=10)
- Group (i) : 10ml of Nanosilver solution for 3 min (n=5)
- Group (ii) : 10ml of Nanosilver solution for 5 min (n=5)

Group F (n=10)
- Group (i) : 10ml of Nanosilver in propylene glycol for 3 min (n=5)
- Group (ii) : 10ml of Nanosilver in propylene glycol for 5 min (n=5)

Step 5 : Tooth Sectioning

After final irrigation, the canals of all sample groups flushed with 0.9% saline. All the samples were removed from wax block and embedded in Acrylic blocks (Fig.24). After acrylic have been set; samples were subjected to Hard tissue microtome(Leica SP 1600) sectioning (Fig.25).

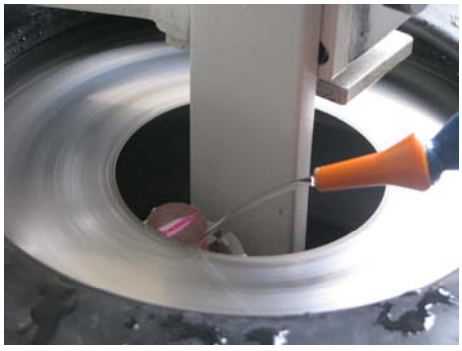
All the root samples were sectioned longitudinally in labio lingual direction under water as coolant to the thickness of 30µ.

(Fig.21) Conjugation of experimental solution with Rhodamine B Dye

(Fig.22) Conjugation of Nanosilver with Rhodamine B and Nanosilver with Propylene Glycol

(Fig.23) Root Canal Irrigation

(Fig.24) Embedding samples in acrylic blocks



(Fig.25) Sectioning of samples with hard tissue microtome

(Fig.26) Sectioned root samples mounted on glass slides

Step 6 : Confocal Image Analysis

Sectioned root samples were placed on the glass slides(Bluestar) and covered with 22x50mm rectangular thin glass coverslip(Bluestar) of 0.17micron thickness and slides were labeled (Fig.26). Samples were then viewed under Confocal Laser Scanning Microscope(Leica TCS SP2, Germany) using argon Laser at an excitation wavelength of 529nm at 20x magnification to analyse the depth of penetration of each irrigant. Images were captured using image capture option in the computer system attached to the Confocal Laser Scanning Microscope. The captured images were qualitatively and quantitatively analysed (Fig.27-36, Graph 1-3)

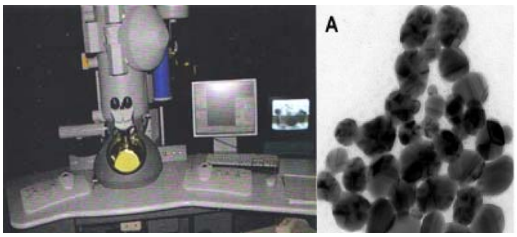
Depth of penetration of each experimental irrigants have been indirectly measured with the aid of Rhodamine dye fluorescence at 3 different levels. i.e. maximum depth of penetration was measured at the coronal 3rd; middle 3rd and apical 3rd. The values have been noted at each level for 5 samples in each subgroups and the mean value have been taken as the max depth of penetration of the irrigant at that particular level in that subgroup. Depth of penetration of all the samples were measured, tabulated and statistically analysed using 'SPSS statistics v 17' software (see Table 1-13 and Diagram 1-6).

PICTORIAL REPRESENTATION OF METHODOLOGY

Step 1

Synthesis of Silver Nanoparticles solution

Step 2



TEM Analysis

Step 3

Dye Dilution

Step 4

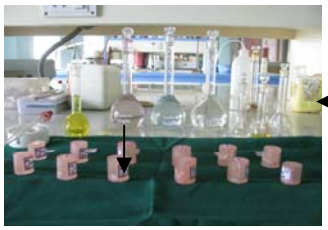
Extracted Teeth

Decoronation

Instrumentation

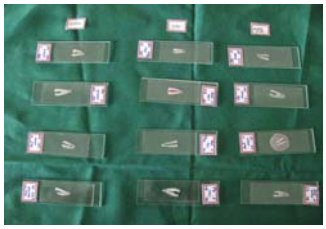
Specimens in Wax blocks

Irrigation

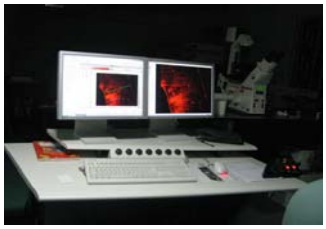


Acrylic blocks

Sectioning



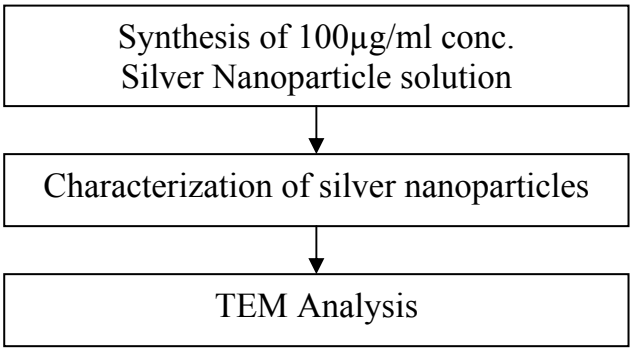
Tissue sections



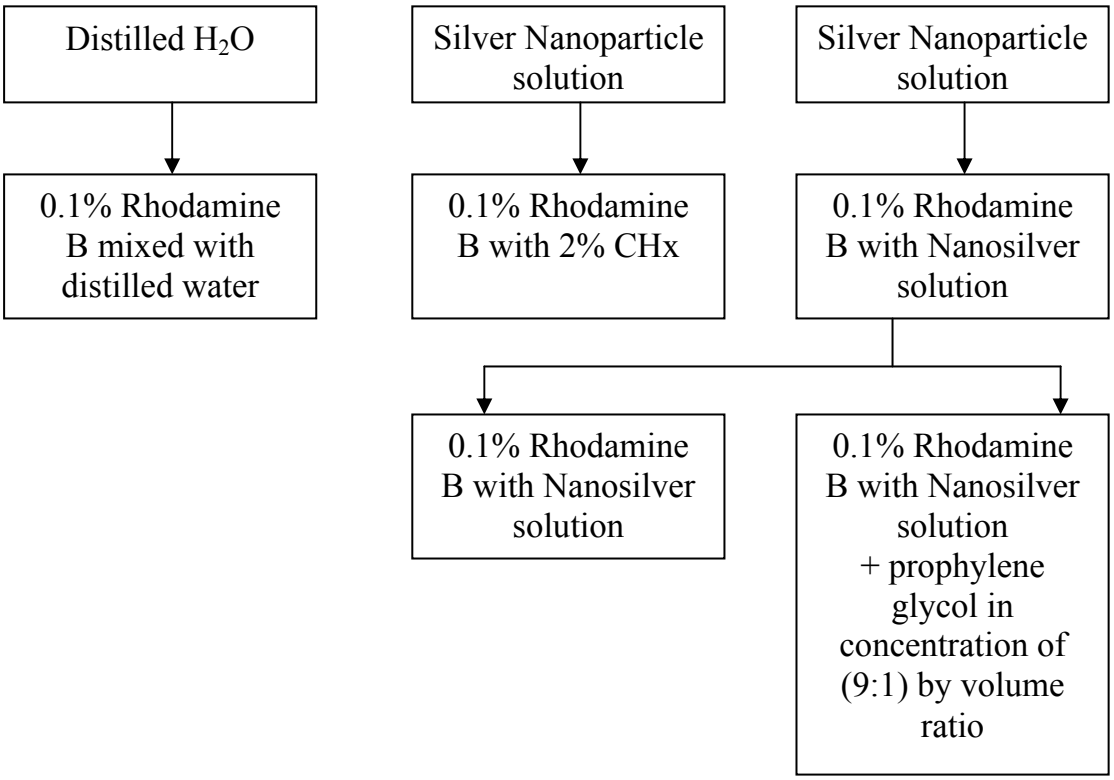
Confocal Microscope Analysis

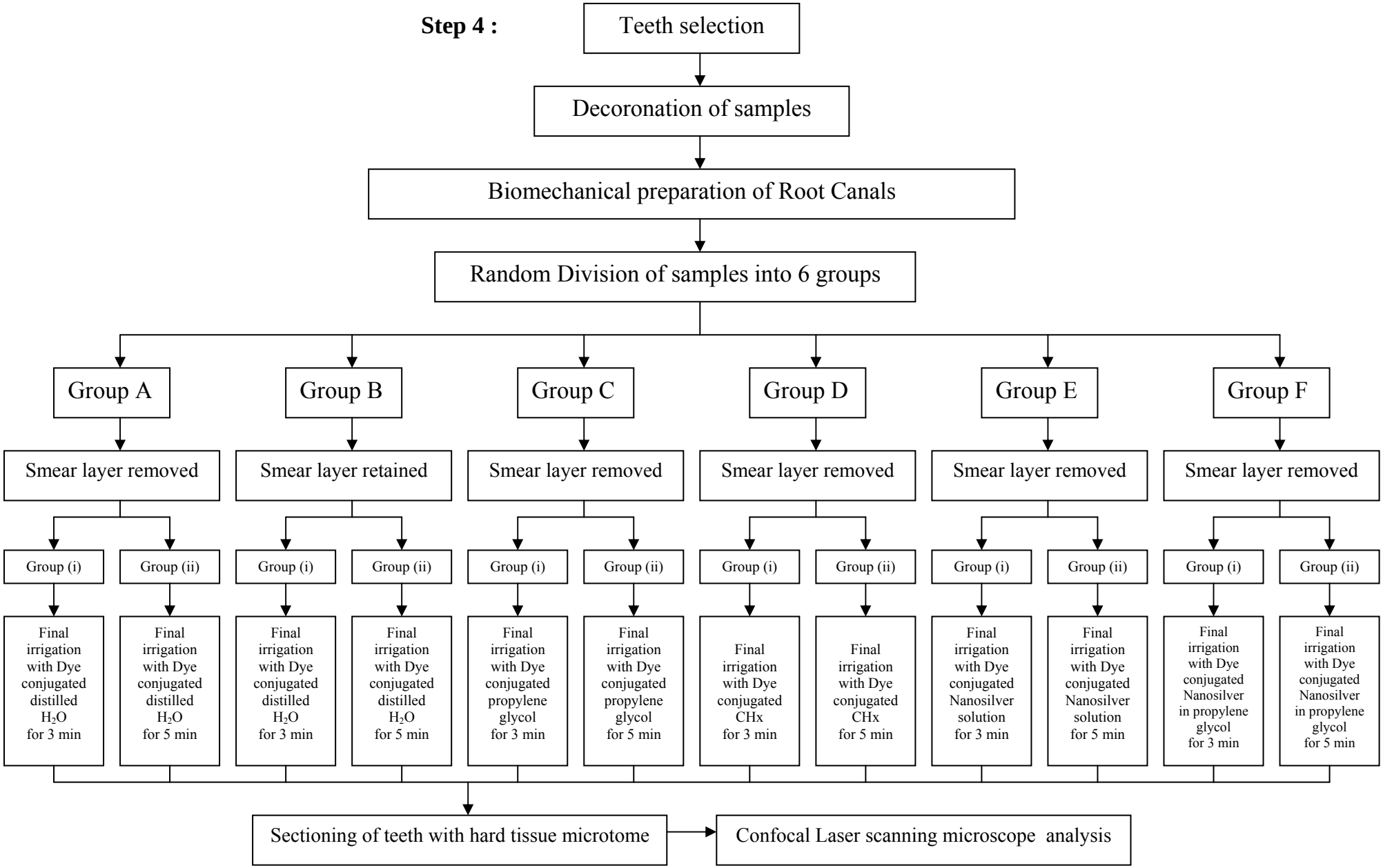
SCHEMATIC REPRESENTATION OF METHODOLOGY

Step 1 & Step 2 : Synthesis of Silver Nanoparticles Solution and its characterization



Step 3 : 0.1% Rhodamine Dye Dilution and conjugation with Experimental solutions




Step 4 :
Teeth selection
Decoronation of samples
Biomechanical preparation of Root Canals
Random Division of samples into 6 groups
Group A
Group B
Group C
Group D
Group E
Group F
Smear layer removed
Smear layer retained
Smear layer removed
Smear layer removed
Smear layer removed
Smear layer removed
Group (i)
Group (ii)
Final irrigation with Dye conjugated distilled H_2O for 3 min
Final irrigation with Dye conjugated distilled H_2O for 5 min
Final irrigation with Dye conjugated distilled H_2O for 3 min
Final irrigation with Dye conjugated distilled H_2O for 5 min
Final irrigation with Dye conjugated propylene glycol for 3 min
Final irrigation with Dye conjugated propylene glycol for 5 min
Final irrigation with Dye conjugated CHx for 3 min
Final irrigation with Dye conjugated CHx for 5 min
Final irrigation with Dye conjugated Nanosilver solution for 3 min
Final irrigation with Dye conjugated Nanosilver solution for 5 min
Final irrigation with Dye conjugated Nanosilver in propylene glycol for 3 min
Final irrigation with Dye conjugated Nanosilver in propylene glycol for 5 min
Sectioning of teeth with hard tissue microtome
Confocal Laser scanning microscope analysis

Results

RESULTS

Table showing maximum penetration depth values of all the groups

(Table 1)

		Group A (+ve control) Distilled H_20 (in µm)					Group B (-ve control) Smear layer + Distilled H_20 (in µm)					Group C (+ve control) Propylene Glycol acqueous solution (in µm)				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
3 min	C	60	61.1	61.3	63.4	59.8	9.4	7.31	6.72	7.95	6.91	724	756.2	659.3	684.4	797.7
	M	62	65.4	63.1	65.1	63.3	6.21	5.28	4.27	5.16	5.4	741.2	778.1	686.4	697.2	814.6
	A	45	42.1	47.9	40.2	43.7	4.24	3.56	2.14	3.20	2.71	452.5	434.5	447.2	394.6	512.4
5 min	C	63	62.1	62.15	60.1	64.7	6.43	7.21	8.24	7.53	7.93	733.6	764.2	672.6	702.6	815.2
	M	65.2	68.5	67.4	65.2	67.2	4.27	5.06	6.19	5.24	6.41	754.7	782.9	683.9	724.8	832.8
	A	50	46.1	47.2	43.1	45.1	2.71	2.43	3.96	3.14	4.32	453.7	514.6	402.7	497.5	517.3

		Group D Chlorhexidine (in µm)					Group E Nanosilver (in µm)					Group F Nanosilver in Propylene glycol (in µm)				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
3 min	C	723.17	521.7	714.1	822.3	643.1	554	601.4	654	572.4	632	819.4	723.7	627.39	504.9	598.3
	M	748.2	557.2	734.2	842.87	657.9	566.4	621.9	667.8	589.3	649.2	827.13	754.2	643.8	532.1	623.7
	A	384.84	421.3	352.4	534.9	414.2	342.1	304.2	423.2	398.1	431.5	478.9	312.5	307.5	314.7	415.8
5 min	C	734.2	553.7	843.5	784.2	672	561	614.1	668.1	589.2	651	850.17	627.39	751.72	573.1	618.9
	M	761.3	595.43	861.4	793.8	684	578.9	637.4	679.8	609.1	666.3	888.26	641.52	778.35	594.6	644.1
	A	402.3	446.51	514.7	486.3	427	355.9	327.1	439.3	411.7	447.3	598.8	427	408.51	383.9	427.9

C – Coronal 3rd M – Middle 3rd A – Apical 3rd

STATISTICAL ANALYSIS

RESULT WAS ANALYSED USING ANNOVA FOLLOWED BY TUKEY HSD TEST

Group A

		Duration		
		3 min Mean / SD	5 min Mean / SD	P value for 3 and 5 min
At different levels	C	61.12 / 1.43	62.41 / 1.67	0.226
	M	63.78 / 1.43	66.70 / 1.46	0.013*
	A	43.78 / 2.92	46.30 / 2.56	0.155
P value for C, M, A		<0.001**	<0.001**	

(Table 2) – Showing mean values & standard deviation of Group A

Note : ** highly significant at 1% level *significant at 5% level

- There is no statistically significant difference between 3 mins & 5 mins
- There is no statistically significant difference at the level C & M
- Depth of penetration is statistically highly significant comparing C Level to A level & M Level to A Level

Group B

		Duration		
		3 min Mean / SD	5 min Mean / SD	P value for 3 and 5 min
At different levels	C	7.26 / 0.48	7.47 / 0.70	0.594
	M	5.26 / 0.69	5.43 / 0.87	0.742
	A	3.17 / 0.80	3.31 / 0.91	0.787
P value for C, M, A		<0.001**	<0.001**	

(Table 3) – Showing mean values & standard deviation of Group B

Note : ** highly significant at 1% level *significant at 5% level

- There is no statistically significant difference between 3 mins & 5 mins
- There is no statistically significant difference at the level C & M
- Depth of penetration is statistically highly significant comparing C Level to A level & M Level to A Level

Group C

		Duration		
		3 min Mean / SD	5 min Mean / SD	P value for 3 and 5 min
At different levels	C	684.87/55.28	717.50/55.22	0.713
	M	708.07/53.99	739.19/56.56	0.734
	A	421.53/42.43	455.36/48.79	0.347
P value for C, M, A		<0.001**	<0.001**	

(Table 4) – Showing mean values & standard deviation of Group C

Note : ** highly significant at 1% level *significant at 5% level

- There is no statistically significant difference between 3 mins & 5 mins
- There is no statistically significant difference at the level C & M
- Depth of penetration is statistically highly significant comparing C Level to A level & M Level to A Level

Group D

		Duration		
		3 min Mean / SD	5 min Mean / SD	P value for 3 and 5 min
At different levels	C	724.32/111.33	737.64111.25	0.655
	M	743.5/106.94	755.82/102.61	0.651
	A	448.24/68.98	477.16/45.21	0.386
P value for C, M, A		<0.001**	<0.001**	

(Table 5) – Showing mean values & standard deviation of Group D

Note : ** highly significant at 1% level *significant at 5% level

- There is no statistically significant difference between 3 mins & 5 mins
- There is no statistically significant difference at the level C & M
- Depth of penetration is statistically highly significant comparing C Level to A level & M Level to A Level

Group E

		Duration		
		3 min Mean / SD	5 min Mean / SD	P value for 3 and 5 min
At different levels	C	602.76/41.18	616.68/43.83	0.619
	M	618.92/41.69	634.30/41.26	0.574
	A	379.82/54.84	396.26/52.70	0.642
P value for C, M, A		<0.001**	<0.001**	

(Table 6) – Showing mean values & standard deviation of Group E

Note : ** highly significant at 1% level *significant at 5% level

- There is no statistically significant difference between 3 mins & 5 mins
- There is no statistically significant difference at the level C & M
- Depth of penetration is statistically highly significant comparing C Level to A level & M Level to A Level

Group F

		Duration		
		3 min Mean / SD	5 min Mean / SD	P value for 3 and 5 min
At different levels	C	654.74/120.68	684.26/113.95	0.701
	M	676.19/115.58	709.37/121.24	0.670
	A	365.88/77.69	449.22/85.51	0.145
P value for C, M, A		<0.001**	<0.001**	

(Table 7) – Showing mean values & standard deviation of Group F

Note : ** highly significant at 1% level *significant at 5% level

- There is no statistically significant difference between 3 mins & 5 mins
- There is no statistically significant difference at the level C & M
- Depth of penetration is statistically highly significant comparing C Level to A level & M Level to A Level

Group D (Vs) Group E (Vs) Group F

	Groups		
Levels	Group D Mean	Group E Mean	Group F Mean
C	730.98	609.72	669.5
M	749.66	626.61	692.78
A	462.7	388.04	407.55
P value	0.097	0.066	0.264

(Table 8) – Showing mean values of Group D, Group E and Group F

Note : ** highly significant at 1% level *significant at 5% level

- Depth of penetration is more in Group D followed by Group F, followed by Group E (D>F>E) (ref Diagram1)
- There is no statistically significant difference at the level C & M

Group C (Vs) Group F

	Groups		
Levels	Group C Mean	Group F Mean	P value
C	701.2	669.5	0.133
M	723.63	692.78	0.166
A	438.45	407.55	0.097

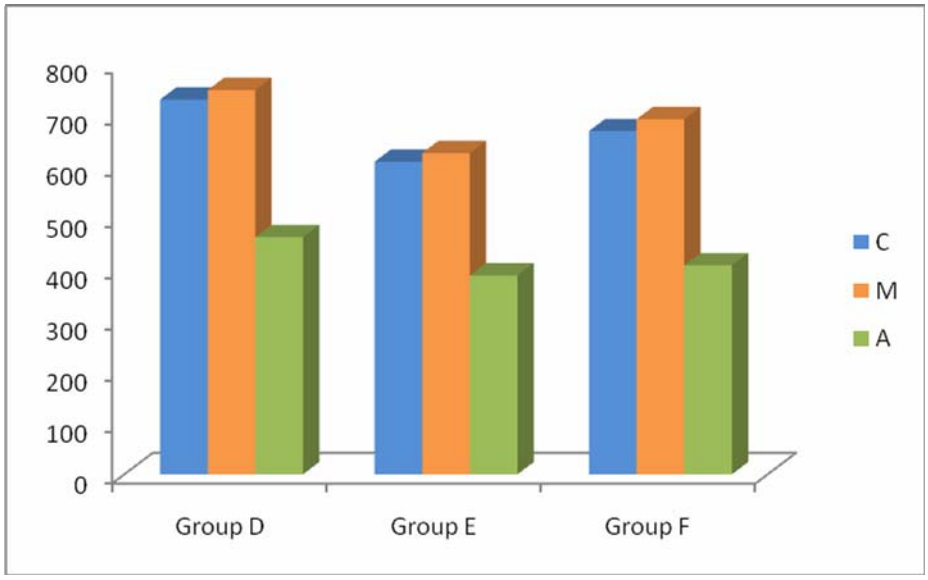
(Table 9) – Showing mean values of Group C and Group F

Note : ** highly significant at 1% level *significant at 5% level

- Depth of penetration is more in Group C followed by Group F (C>F) (ref Diagram 2)
- There is no statistically significant difference at the level C & M

Comparing Group D (Vs) Group E (Vs) Group F

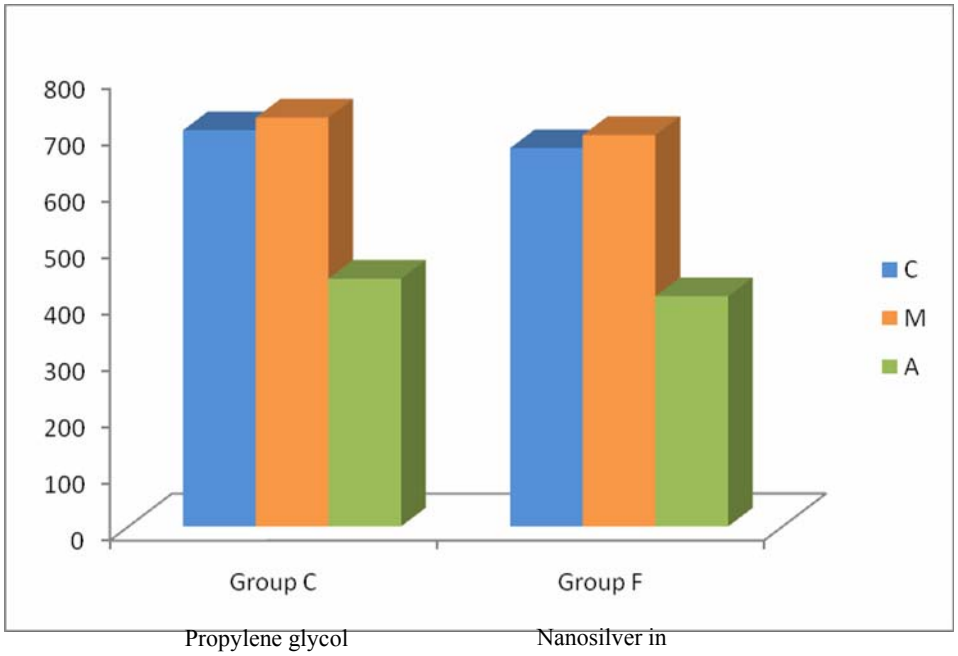
(Diagram 1)



CHx Nanosilvcr Nanosilver in propylene glycol

Comparing Group C (Vs) Group F

(Diagram 2)



Propylene glycol Nanosilver in propylene glycol

Group C (Vs) Group D

	Groups		
Levels	Group C Mean	Group D Mean	P value
C	701.2	730.98	0.437
M	723.63	749.66	0.476
A	438.45	462.7	0.312

(Table 10) – Showing mean values of Group C and Group D

Note : ** highly significant at 1% level *significant at 5% level

- Depth of penetration is more in Group D followed by Group C (D>C) (ref Diagram3)
- There is no statistically significant difference at the level C & M

Group A (Vs) Group D (Vs) Group E

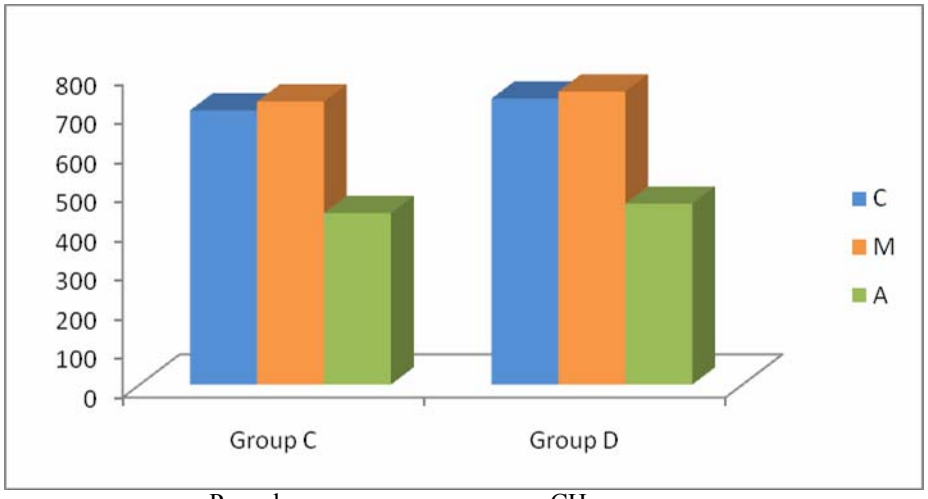
	Groups		
Levels	Group A Mean	Group D Mean	Group E Mean
C	61.77	730.98	609.72
M	65.24	749.66	626.61
A	45.04	462.7	388.04
P value	<0.001**	<0.001**	<0.001**

(Table 11) – Showing mean values of Group A, Group D and Group E

Note : ** highly significant at 1% level *significant at 5% level

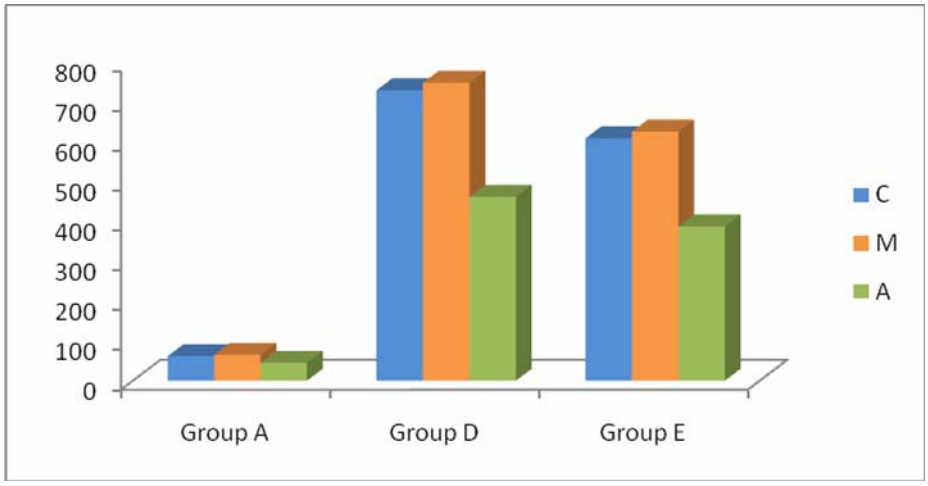
- Depth of penetration is more in Group D followed by Group E, followed by Group A (D>E>A) (ref Diagram 4)
- There is no statistically significant difference at the level C & M
- There is statistically significant difference at C level to A level, M level to A level in group A,D & E

(Diagram 3) Comparing Group C (Vs) Group D



Propylene glycol CHx

(Diagram 4) Comparing Group A (Vs) Group D (Vs) Group E



Distilled water CHx Nanosilver

Table 12

Means Values of all the groups at 3 min duration

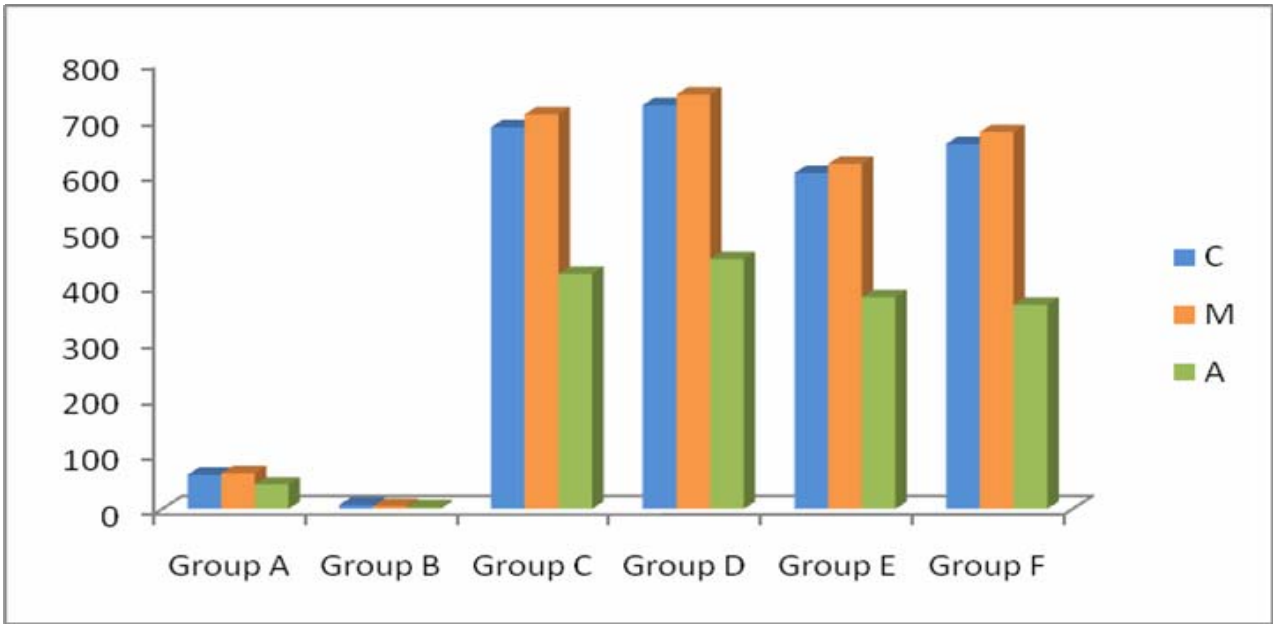
Levels	Group A	Group B	Group C	Group D	Group E	Group F
C	61.12	7.26	684.87	724.32	602.76	654.74
M	63.78	5.26	708.07	743.5	618.92	676.13
A	43.78	3.17	421.53	448.24	379.82	365.88

Table 13

Means Values of all the groups at 5 min duration

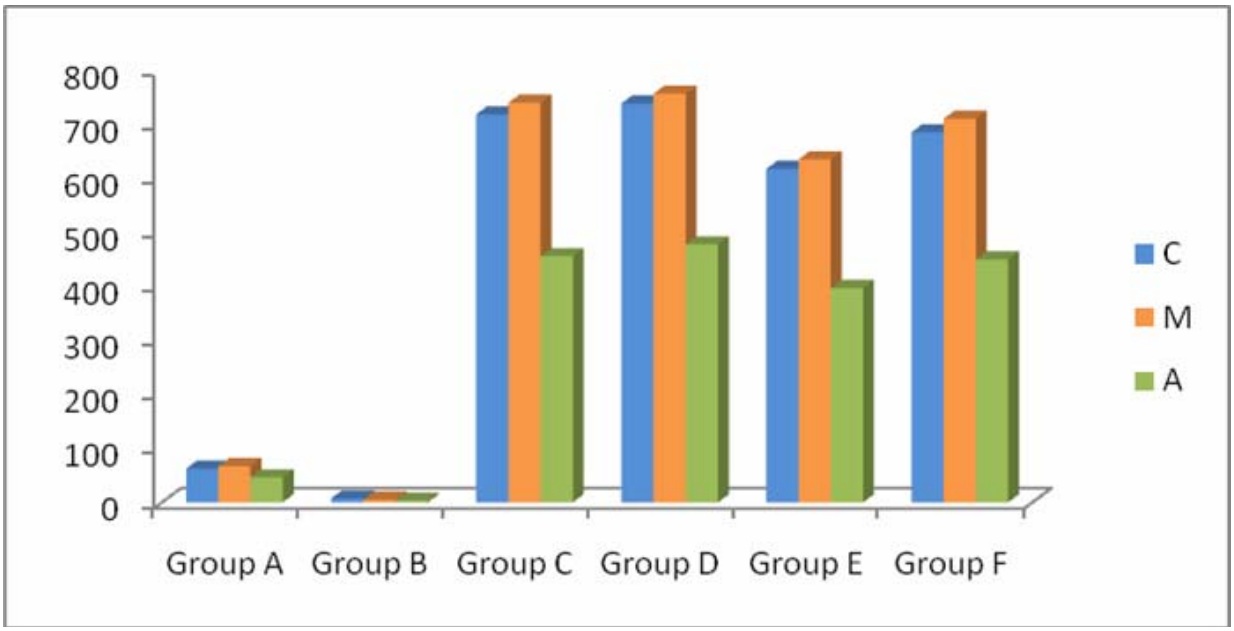
Levels	Group A	Group B	Group C	Group D	Group E	Group F
C	62.41	7.47	717.50	737.64	616.68	684.26
M	66.70	5.43	739.19	755.82	634.30	709.37
A	46.30	3.31	455.36	477.16	396.26	449.22

Diagram 5 – Comparison the depth of penetration of different experimental irrigants at 3 mins



Distilled water | Negative group | Propylene Glycol | CHx | Nanosilver | Nanosilver in propylene glycol

(Diagram 6) – Comparing the mean depth of penetration of all the group irrigants at 5 mins



Distilled water | Negative group | Propylene Glycol | CHx | Nanosilver | Nanosilver in propylene glycol

RESULT ANALYSIS

- Comparing within each groups at different levels, there is no statistically significant difference in depth of penetration at coronal and middle 3rd.

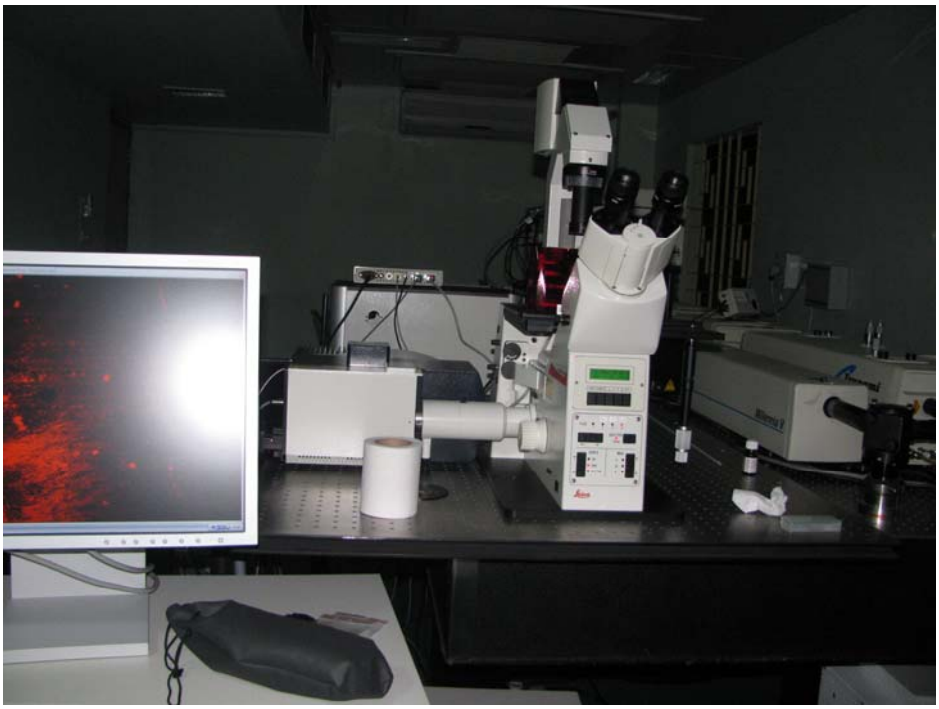
- But there is highly significant difference in penetration depth at apical 3rd in all the groups

- In all the groups there is no significant difference in penetration depth at 3 mins and 5 mins group.

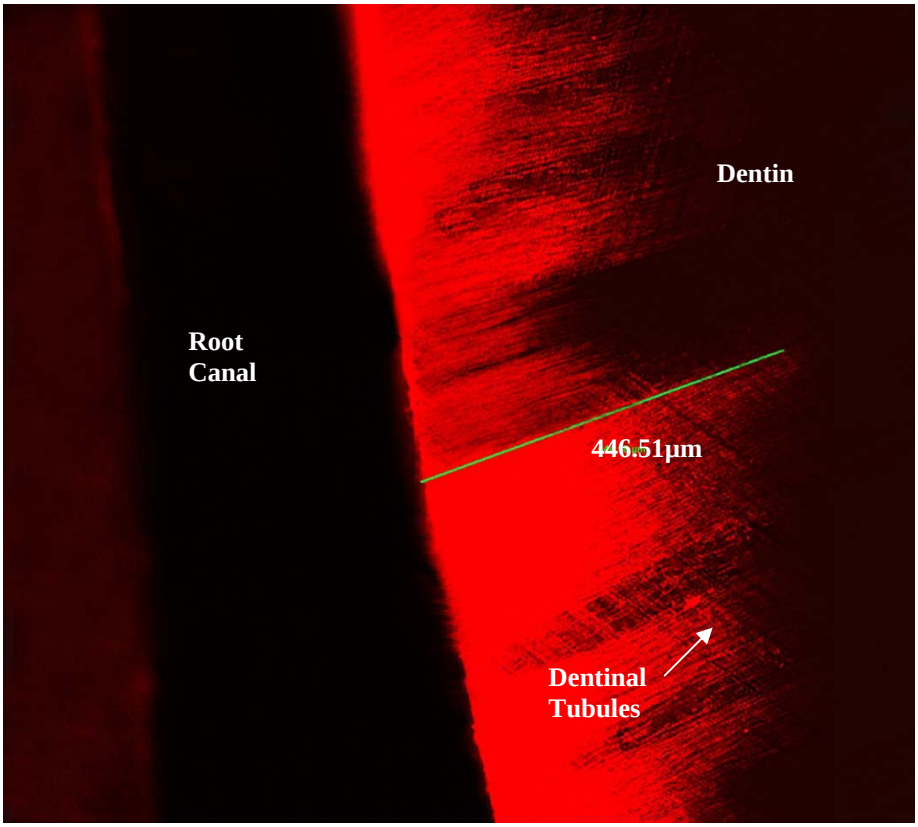
- Overall comparing all the groups Chlorhexidine shows maximum depth of penetration,then propylene glycol, nanosilver in propylene glycol which is then followed by aqueous solution of Nanosilver

  (CHx > propylene glycol > Nanosilver in propylene glycol > Aqueous solution of Nanosilver).

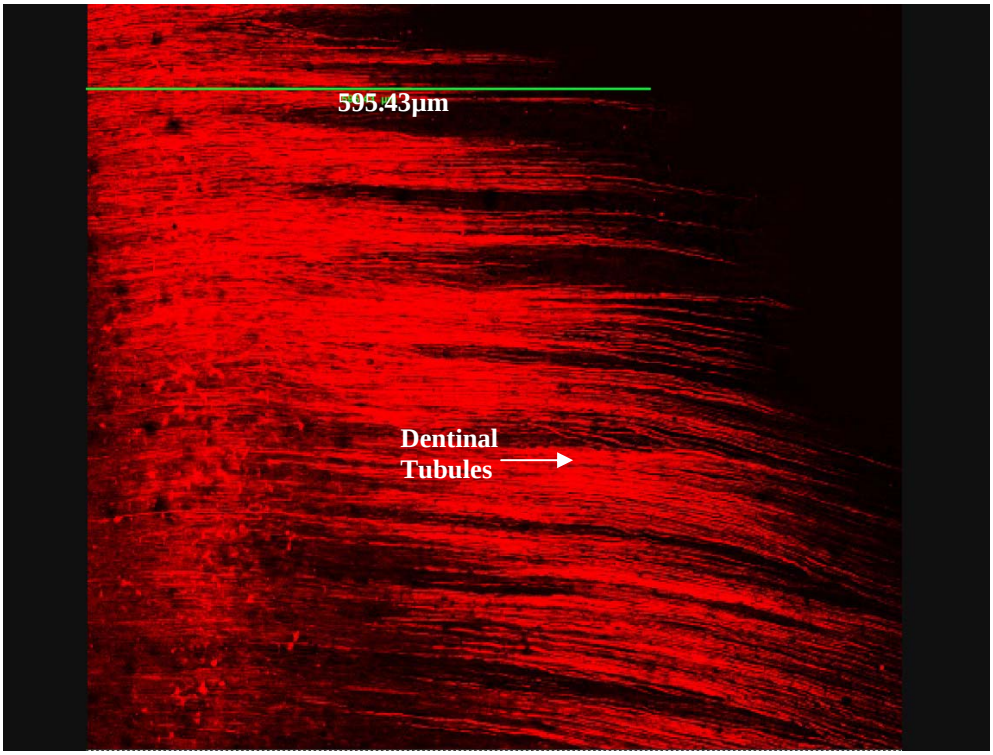
- Negative control group i.e. smear layer retained group showed least irrigant penetration both at 3 min and 5 min duration.



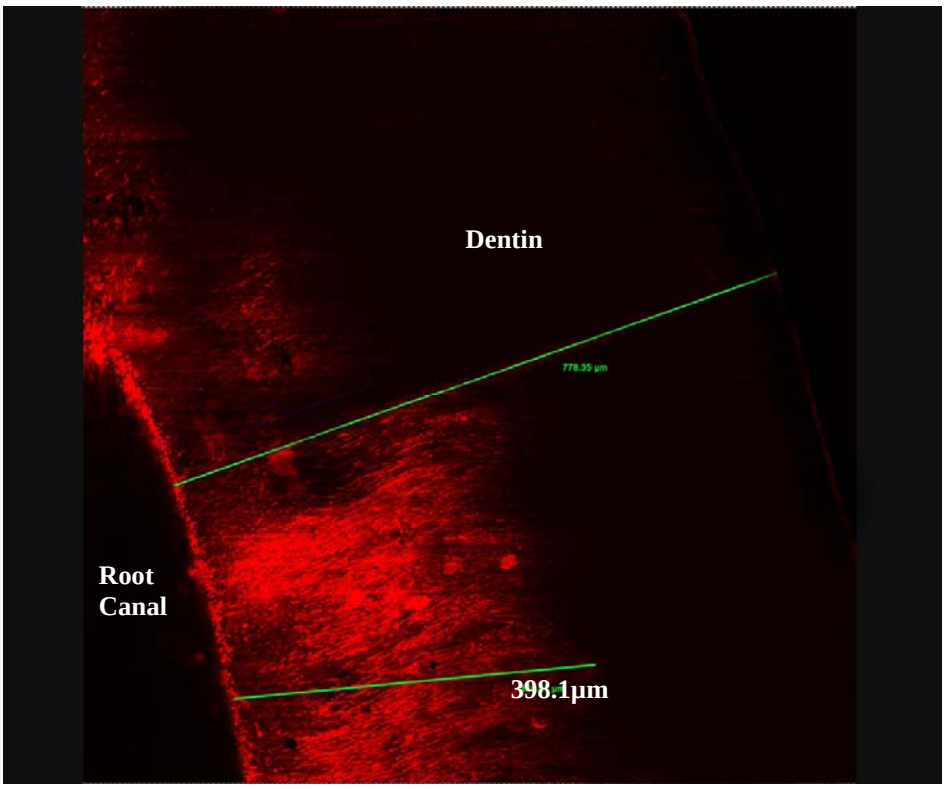
(Fig.27) Close-up View of Confocal Laser Scanning Microscope



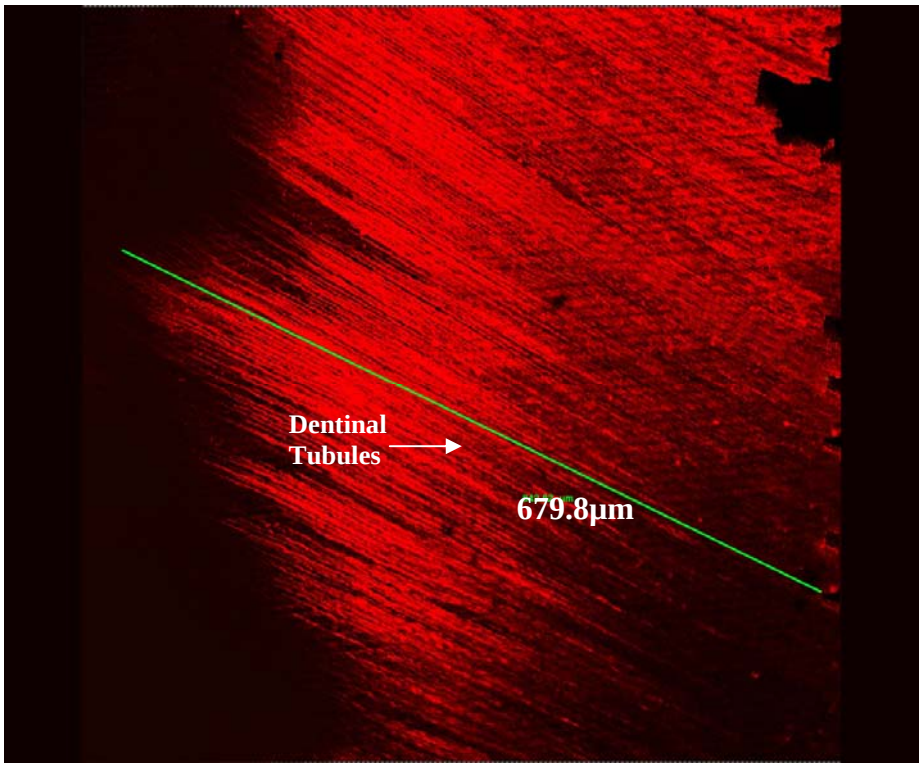
(Fig.28) Depth of penetration of Chlorexidine at Apical 3rd



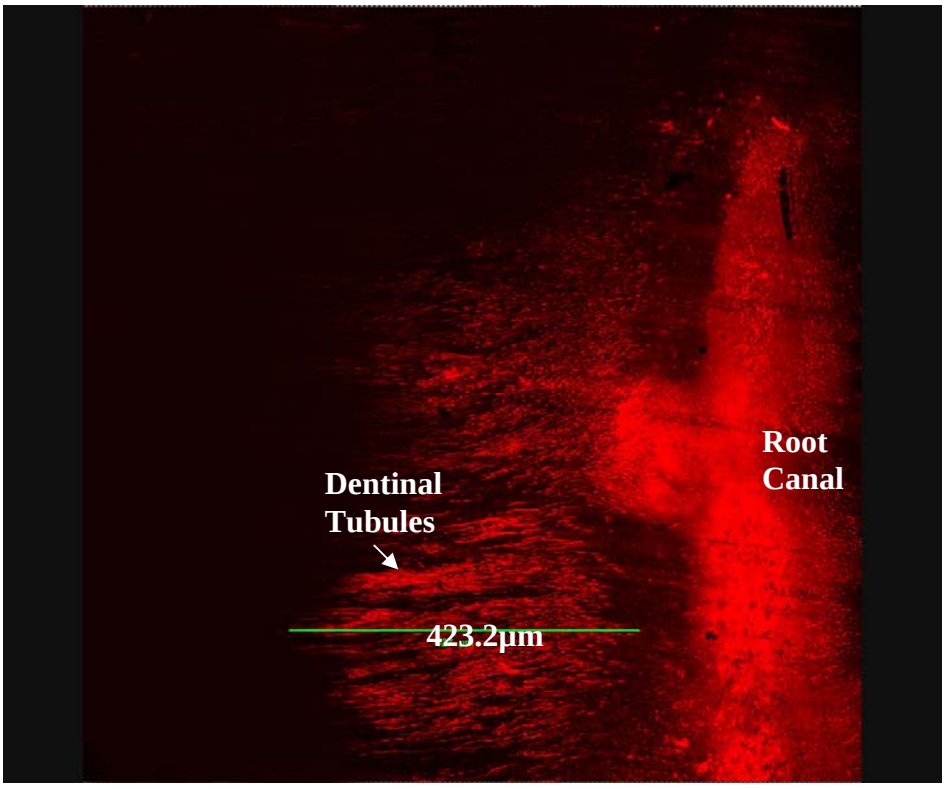
(Fig.29) Depth of penetration of Chlorexidine at Middle and Apical 3rd



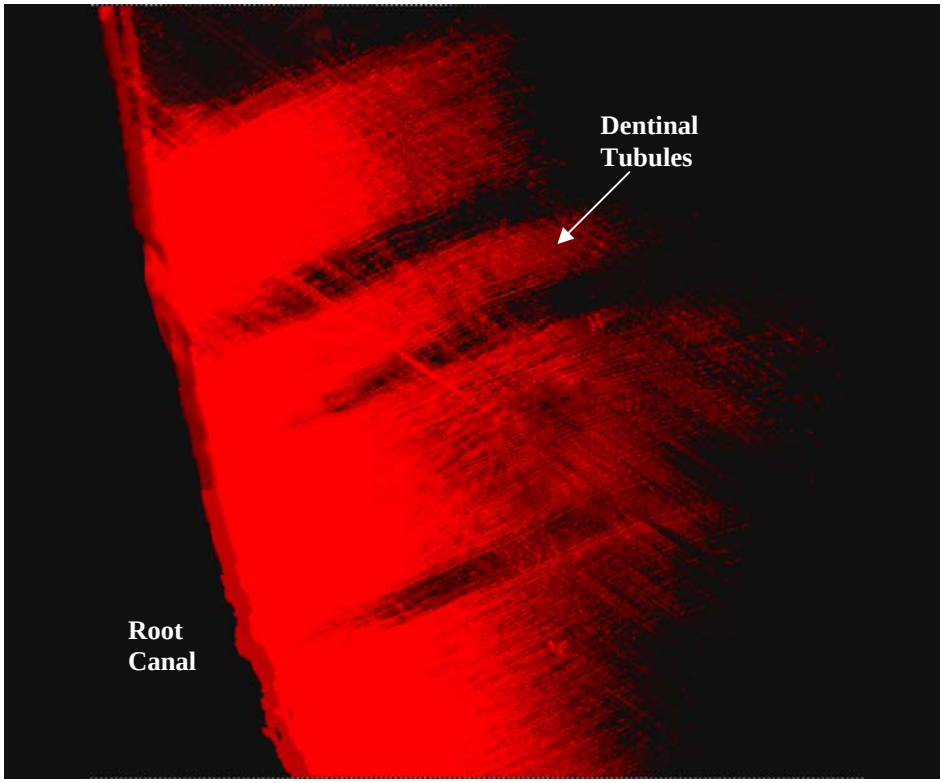
(Fig.30) Depth of penetration of Nanosilver at Apical 3rd



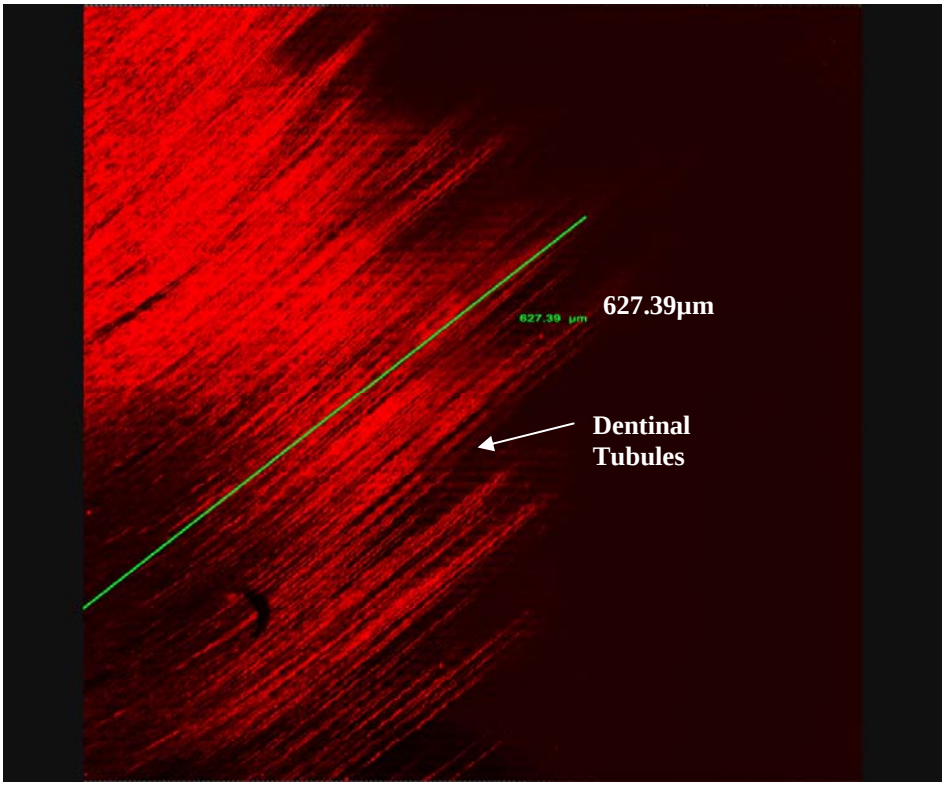
(Fig.31) Depth of penetration of Nanosilver at Middle 3rd



(Fig.32) Depth of penetration of Nanosilver at Apical 3rd

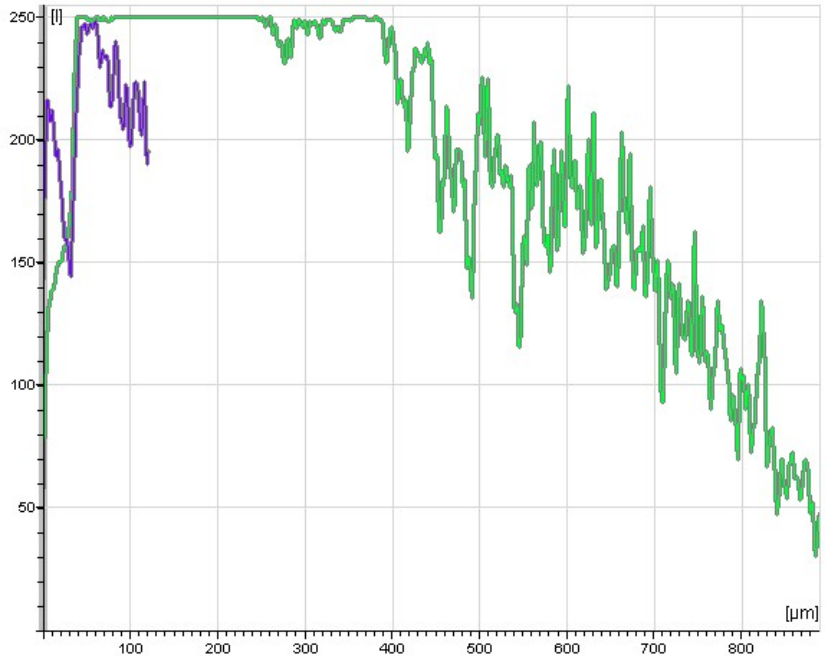


(Fig.33) Depth of penetration of Propylene glycol at middle 3rd



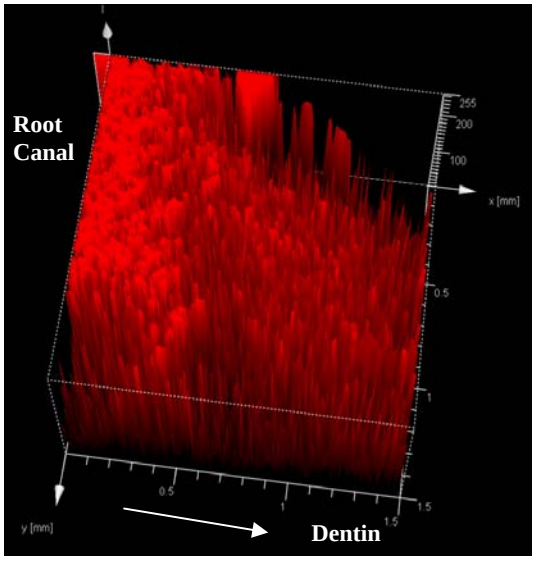
(Fig.34) Depth of penetration of Nanosilver in Propylene glycol at middle 3rd

Graph 1 :



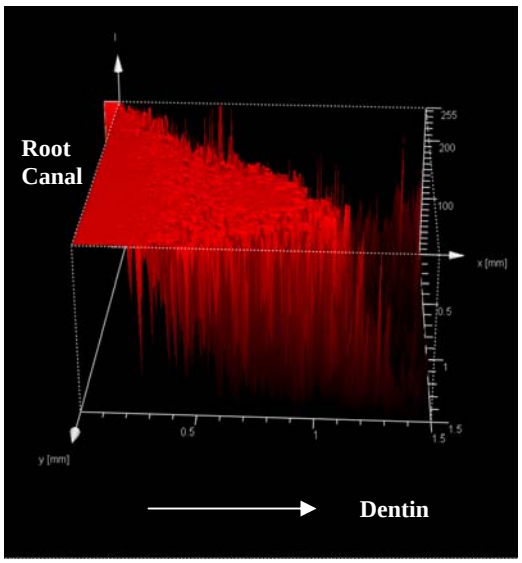
Graph showing the declining intensity of fluorescence of Rhodamine Dye as it moves far away from pulpal end towards DEJ

Graph 2 :

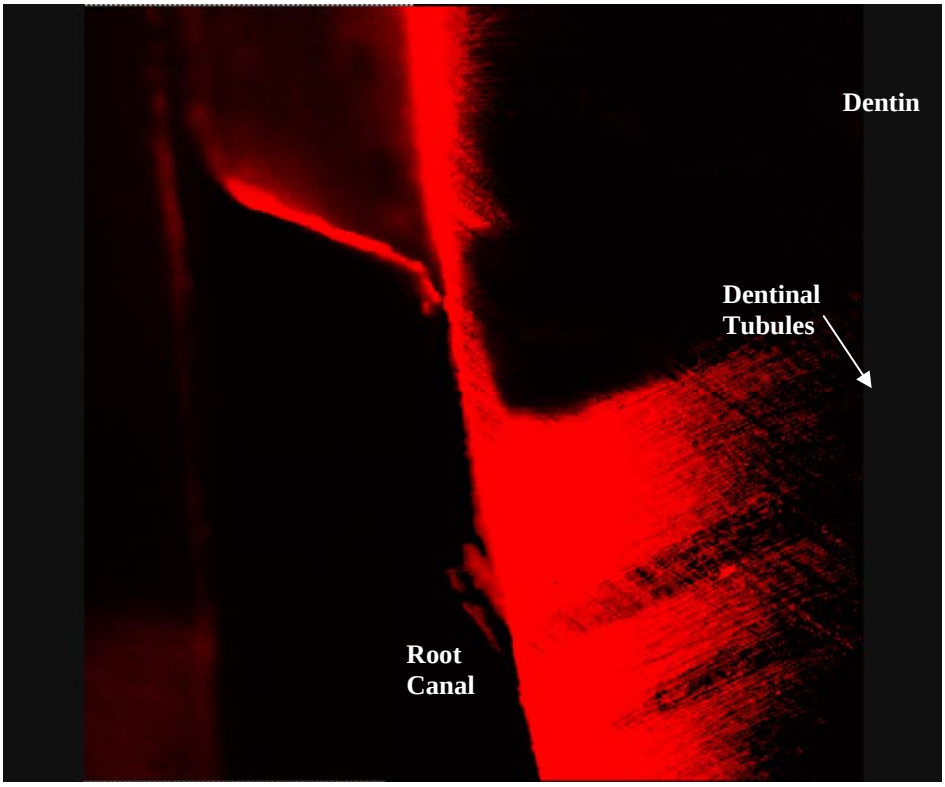


Confocal micrograph revealing the variation in intensity of fluorescence from Z axis point of view

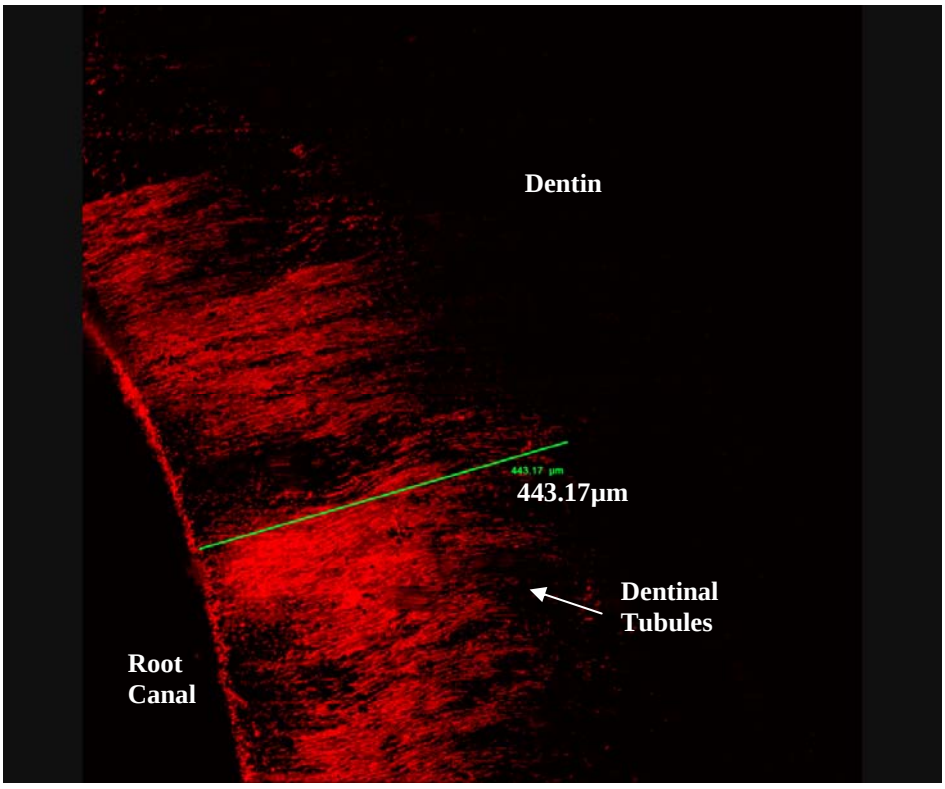
Graph 3 :



Confocal micrograph revealing the variation in intensity of fluorescence at XY axis



(Fig. 35) Depth of penetration of distilled water



(Fig. 36) Depth of penetration of Propylene Glycol at Apical 3rd

Discussion

DISCUSSION

E.faecalis can penetrate from the canal as far as 250µm into the dentinal tubules. It is one of the most resistant micro organisms found in the infected root canal [42,35] and it has been reported that cases refractory to endodontic treatment were associated with this bacterial strain[66,38,70].

In the present study, 2% chlorhexidine was used because invitro studies reports that chlorhexidine completely inhibited the growth of E.faecalis[21]. Recent reports confirms that chlorhexidine has a marked effect against E.faecalis[5,11,33]. Furthermore Chlorhexidine have proved to be effective even at low concentrations against the microorganisms most frequently present in infected canals, anaerobic bacteria and candida albicans[60]. Apart from the positive antimicrobial efficacy of chlorhexidine, it has got substantivity. Because of its cationic properties, chlorhexidine can bind to the hydroxyapatite of the dentine[52] and a gradual release of this bound chlorhexidine may protect the canal against microbial colonization beyond the actual medication period[4].

The course of the dentinal tubules follows a gentle curve in the crown, less so in the root, where it resembles a gentle S (sigmoid curve) in shape. The tubules are longer than the dentin thickness because they curve through dentin. The dentin thickness ranges from 3-10mm or more.

Dentin thickness varied not only from tooth to tooth of same person but also in different surfaces of the same tooth. The buccal surfaces showed maximum thickness followed by lingual and there was no difference in thickness between mesial and distal surfaces. Tubule diameter are larger near the pulpal cavity (3 to 4μm) and smaller at their outer end (1μm). There are more tubules per unit area in the crown than in the root[41,72].

The mean number of dentinal tubules in the middle part of the root was significantly lower than in the coronal portion[73].

In his study on dentinal tubule penetration of R.C.sealer after R.C.dressing with $Ca(OH)_2$, **Semra Calt et.al.T**[58]observed that sealer penetration was deepest at the middle 3rd of the root canal.

Sawnders et al[55] **and vassilliadis et al** [75] reported similar findings. These investigators[55,75]explained that the tubule diameter at the middle 3rd of the root are large enough than colonal 3rd because of the lesser density of the tubules.

Further it has been reported that in the absence of smear layer, tubular penetration is influenced by the physical and chemical properties of material that is intended to be used for its antimicrobial action[19].

Many studies have shown that bacteria may invade the dentinal tubules and accessory canals, wherein they are protected from the action of both instrumentation and irrigants that are only active on the surface[51,70]. A possible cause of failure in treatment is thus the persistence of an endodontic infection sustained by bacteria that have colonized the dentinal tubules[54].

The bactericidal effectiveness of irrigants obviously depends on their ability to penetrate the infected dentinal tubules, a process that is clearly influenced by the presence or absence of a smear layer. It has been shown that the formation of this smear layer reduces root dentin permeability by between 25% - 49%[19]. Thus, the smear layer produced during instrumentation occludes the dentinal tubules and protects the micro organisms from the action of antimicrobial agents. Alternating use of 2.5% sodium hypochlorite and 17% EDTA has been found to be extremely effective to remove the smear layer and keep the dentinal tubules open and patent to the antimicrobial action of the irrigants.

Many studies have been carried out evaluate the efficacy of root canal irrigants. **Elio Berutli et.al** in 1997 studied on penetration ability of different irrigants into Dentinal tubules. In his study he evaluated the depth of penetration of the sodium hypochlorite indirectly based on the presence or absence of the E.faecalis in the depth of the tubules by taking the histologic sections of the samples and viewing under light microscope. The result showed that few dentinal tubules were perfectly clean and bacteria free for their

entire length at the coronal and middle 3rd of the canal. It was hypothesized that the regularity and larger size of the dentinal tubules in this area and greater efficiency of the irrigants cleared out the dentinal tubules more thoroughly in these portions of the root canal.

Richard Buck. et.al.(51) studied about disinfection of Dentinal tubules by various endodontic irrigants. His study demonstrated that irrigants such as NaOCl, RC prep, Betadine and Chlorhexidine are able to penetrate well into the dentinal tubules, he also concluded that all the irrigants penetrated within the tubules in a sufficient concentration to kill all the bacteria.

Kouvao.v et.al.(30) have studied the influence of smear layer on depth of penetration of endodontic sealers.

There are studies evaluating the efficacy of solutions to remove the smear layer by measuring the depth of smear layer removed inside the tubules(14).

There are many studies reporting depth of penetration of sealers, depth of removal of smear layer and depth of penetration of microorganisms into the dentinal tubules. There is no literature or study evaluating the efficacy of the irrigants based on its depth of penetration into the dentinal tubules.

This study is a preliminary step, in which penetrating potential of chlorhexidine and Nanosilver solution have been evaluated with the aid of 0.1m.mol Rhodamine B dye as marker under confocal microscope. Hence a pilot study has been carried out to see the outcome result of the study. Based on the successful result of pilot study, this main study has been carried out.

In this study, teeth extracted from the patients of 15-40 age group have been selected to eliminate influence of age on penetration. As age advances, the dentinal tubule diameter decreases due to dentinal sclerosis[43].

Rhodamine B dye solution[49] at the concentration of 0.1m.mol is used as a marker to evaluate the depth of penetration of irrigants viewed under confocal laser scanning microscope. There is no visible chemical reaction observed when dissolving Rhodamine-B powder with CHx or Nanosilver solution Rhodamine-B is nothing to do with antibacterial efficacy of the CHx or Nanosilver solution because this dye is used just to enable the visibility of the irrigant inside the tubule, otherwise it is nothing to do with clinical application of irrigants. CHx is chosen as an irrigant because of its well studied potential action on persistant, resistance, tubule invading bacteria E-Faecalis. Recent studies have reported on well known action of Nanosilver solution on gram negative bacteria E.coli and E.faecalis[1]. Therefore Nanosilver solution has been included in this comparative invitro study. In order to assess the penetration ability of the dye by its own, aqueous solution of plain Rhodamine-B is used as a control group. Samples are instrumented upto 30 file size so that

the irrigants can reach upto the apical 3rd of the root. Smear layer is removed by alternative irrigation with 17% EDTA and 2.5% NaOCl. Passive irrigation using syringe and 27 gauge needle is the protocol followed in this study. Before irrigation root samples were embedded in the wax blocks to avoid dye penetration into the tubule from the outer end of the tubule in gap junction cases at CEJ and also to avoid apical extrusion of dye and thereby giving false results.

The result of this study reveals that, when comparing within each group at different levels, there is no statistically significant difference in depth of penetration at the coronal and middle 3rd of the root in all the groups (ref. table 2-7). But there is statistically significant difference in penetration depth when comparing coronal & middle 3rd to apical 3rd level (Diagram 1-6). There is an abrupt decrease in the penetration depth at the apical 3rd level in all the groups. The difference could be attributed to the lower number of patent tubules in the apical region because of dentinal sclerosis which is always more advanced in this region[44], inefficient access to this region for irrigants to flushout the debris and remove smear layer to keep the tubules patent.

There is no statistically significant difference in depth of penetration at 3 min and 5 min duration in all the groups. In all the groups, maximum depth of penetration is observed at the middle 3rd level (Diagram 1-6). This might be attributed to the factor that tubule density decreases from the coronal to the apical 3rd, the diameter of the tubules are more at the middle 3rd and this region is well accessible to the flushing action of irrigants and smear layer removal.

Comparing all the groups , CHx showed the maximum depth of penetration followed by the surfactant propylene glycol which is then followed by nanosilver in propylene glycol solution (Table 12,13, Diagram 5,6). This may be attributed to the fact that CHx has less surface tension than Nanosilver solution.

Generally, depth of penetration of an irrigant depends on following factors :

- Patency of dentinal tubules
- Presence or absence of smear layer
- Diameter and orientation of the tubules
- Surface free energy of the tubules
- Wettability or surface tension of the solution
- Irrigation method
- Force with which irrigation has done.

- Decrease in surface free energy => increases surface tension, increases the contact angle and decreases the wettability.
- Decrease in free surface energy decreases the spreading and thus reduces the adhesion of the material.
- Smear layer found to have high surface tension, but it act as a physical barrier to the diffusion of the irrigants. By occluding the dentinal tubules, the permeability of dentin would be reduced and thereby microcapillary penetration of an irrigant.

- Diameter and size of open dentinal tubules increased after NaOCl treatment of demineralized dentin.

Both chelating agents and NaOCl decreases the wettability of dentine surface. Therefore for the optimal penetration, surface tension of an irrigant should be low or surface tension reducing agents i.e surfactant should be added.

So here in this study propylene glycol has been added to the Nanosilver solution to reduce the surface tension[10]. Propylene glycol is an inert surfactant used commonly in cosmetics like soap, creams, shampoo..etc and in pharmaceutical drugs. To evaluate the penetrating potential of propylene glycol alone, plain propylene glycol solution mixed with dye is used as control group.

The result of this study showed that propylene glycol is as efficient as penetrating potential of chlorhexidine. CHx group and Nanosilver in propylene group showed effective result. Nanosilver in propylene glycol is slightly less penetrated than CHx and propylene glycol but the difference is not statistically significant (Table 8, Diagram 1).Propylene glycol is an inert surfactant which doesn't have any antibacterial property.

Therefore we can conclude that Nanosilver solution can be used as an alternative to CHx as Nanosilver has broad spectrum of action particularly on gram –ve organisms like E.faecalis, better penetrating ability more or less equal to CHx and efficient increase in antibacterial action due to increase in surface area of Nanoscale size of the silver particles. But further studies are required to evaluate the substantivity of Nanosilver solution.

Summary

SUMMARY

Silver Nanoparticles in the concentration of 100mg/ml prepared by Borohydride reduction method and were characterized by TEM. Sixty freshly extracted single rooted and single canal teeth were selected and stored in 0.9% saline. Teeth were decoronated, pulp extirpated and patency of the canals were checked with No.10 k file. Working length determined and Instrumentation of the canal done with K-files upto No. 20 file size and then the apical 3rd of the canals are enlarged using rotary protaper file upto 30 file size (F3). All the samples are embedded in wax blocks. Samples are then randomly divided into 6 main groups containing 10 samples in each group. Six groups are further subdivided into Group (i) & (ii) wherein each subgroup containing 5 samples.

Smear layer is removed in all the main groups except group B by alternating irrigation with 17% EDTA and 2.5% NaOCl. Then the canal is flushed with 0.9% saline. Before final irrigation, 0.1% of Rhodamine-B is added to all the irrigants used in this study i.e. distilled H_20, propylene glycol, CHx, Nanosilver solution and Nanosilver in propylene glycol (9:1) by vol.

Group A (n=10) (positive control group) → Final irrigation done with Distilled H_20 containing 0.1% Rhodamine-B subgroup(i) is irrigated for 3 min and group(ii) for 5 min.

Group B (n=10) (negative control group) → Smear layer retained and final irrigation done with distilled water containing 0.1% Rhodamine B.

(5n) group (i) → irrigated for 3 min

(5n) group (ii) → irrigated for 5 min

Group C (n=10) (positive control) – propylene glycol solution containing 0.1% Rhodamine B

(5n) group (i) → irrigation done for 3 min

(5n) group (ii) → irrigation done for 5 min

Group D (n=10) Final irrigation done with CHx solution containing 0.1% Rhodamine B.

(5n) group (i) → for 3 min

(5n) group (ii) → for 5 min

Group E (N=10) Irrigated with Nanosilver solution containing 0.1% Rhodamine B

(5n) group (i) → for 3 min

(5n) group (ii) → irrigated for 5 min

Group F (n=10) Final irrigation done with (9:1 vol) Nanosilver in propylene glycol mixed with 0.1% Rhodamine B

group (i) (5n) → Irrigated for 3 min

group (ii) (5n) → Irrigated for 5 min

After final irrigation all the samples were flushed with 0.9% saline. Samples were removed from wax block and mounted horizontally on Acrylic block for sectioning. All the samples sectioned longitudinally with hard tissue microtome. The sectioned samples were viewed under confocal laser scanning microscope and maximum depth of penetration of the irrigant was measured at coronal 3rd, middle 3rd and apical 3rd of the root.

All the values are tabulated and statistically analysed using ANNOVA followed by HSD test.

Conclusion

CONCLUSION

From the study, we conclude the following :

1. Chlorhexidine showed the maximum depth of penetration followed by propylene glycol, Nanosilver in propylene glycol, Nanosilver aqueous solution which is then followed by Control group irrigants. [CHx > propylene glycol > Nanosilver in propylene glycol > Nanosilver > Distilled water > negative control group (Group B)].

2. In this study, Smear layer retained sample showed least depth of penetration. This shows that smear layer acts as a barrier for the penetration of the irrigant.

3. There is no statistically significant difference in depth of penetration by irrigating for 3 min or 5 min duration in all the groups.

4. comparing the depth of penetration of the experimental irrigants at the level of coronal, middle and apical 3^{rd} => middle 3^{rd} showed the maximum depth of penetration followed by coronal 3^{rd} and least at the apical 3^{rd} in all the groups.[M > C > A]

This study suggests that Nanosilver solution with addition of Propylene glycol (Surfactant) can be used as an alternative to 2% Chlorhexidine solution against Micro organisms that invades dentinal tubules and cause failure of Root canal treatment especially gram negative organisms like E.faecalis.

Further, evaluation of substantivity of Nanosilver solution, effect of addition of propylene glycol on bactericidal concentration of nanosilver and invivo clinical trials have to be carried out before clinical application of Nanosilver solution as an irrigant.

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